THE EFFECT OF SOIL NUTRIENT AVAILABILITY ON RESOURCE ALLOCATION IN TROPICAL TREE SPECIES

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

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ABSTRACT

Tropical tree communities are diverse in both their taxonomic composition and in their functional strategies for resource use and acquisition. Soil resources influence productivity of tropical forests and the distributions of tree species across edaphically heterogeneous landscapes. My dissertation evaluates functional variation among tropical trees in nutrient use, and the implications of this functional diversity of tropical forest nutrient and carbon cycling. Through a comparison forest litterfall patterns along a natural fertility gradient and experimental nutrient addition, I show that responses of forest productivity to nutrient availability are difficult to predict across space from environmental parameters alone due to turnover in forest functional composition. Evaluation of wood and foliar nutrient allocation along a soil fertility gradient demonstrates that tree species vary enormously both within and among soil habitats in the allocation of soil-derived nutrients to both foliar and woody biomass. I investigated the function of wood nitrogen and phosphorus repositories by conducting a sapling defoliation experiment, finding that wood P reserves are a dynamic pool reflecting both P in the soil and the demand for P allocation to leaves. Finally, I found that soil phosphorus availability is strongly correlated with the frequency of multiple stemmed trees across a regional forest plot network, indicating that reserves of soil derived nutrients influence the survival of trees after damage. By uncovering the importance of wood nutrient storage in forest ecosystem and community dynamics, this dissertation highlights a novel mechanism by which soil fertility influences the structure and function of tropical forests.

INDEX WORDS: tropical forest, nutrient limitation, nitrogen, phosphorus, environmental gradients, functional traits, resource-use efficiency, resprouting

ACKNOWLEDGMENTS

This research would not have been possible without countless people, places, and plants. First, I thank my advisor Jim Dalling for supporting my research and introducing me to Fortuna, one of my favorite places in the world. It has been a pleasure to benefit from Jim's insight and kindness, and I look forward our future collaborations. I am grateful to the wonderful women of the Dalling lab, including Kelly Andersen, for pioneering field work at Fortuna, Claire Baldeck, for being my number one science role model, Pimonrat Tiansawat, for radiating support and positivity, Cecilia Prada, for being an excellent botanist and better friend, and Jennifer Jones, for bringing enthusiasm to every situation, even bark grinding. I am especially grateful to my lab twin, Adriana Corrales, for sharing this journey with me and teaching me which mushrooms are safe to eat with mac and cheese.

Stipend funding for this project was provided NSF Graduate Research Fellowship and Illinois Distinguished Fellowship. Research funding was provided by NSF Doctoral Dissertation Improvement Grant (DEB-1311379), and a number of grants from the University of Illinois including the Program for Ecology and Evolutionary Ecology Summer Research Grant, Clark Research Support Grant, Tinker Summer Research Fellowship, and School of Integrative Biology Special Funds.

Fieldwork for this project could not have been accomplished without an army of field helpers. In particular, I appreciate the expertise and patience of my long time Panamanian field assistants, Carmen Velasquez and Fredy Miranda. I am grateful to Arturo Morris for identifying trees for my study. I thank Professor Pedro Cabellero and his students from the Universidad Autónoma de Chiriquí, who made year round data collection at Fortuna possible. I thank my cousin Rob Heineman for being a cheerful summer intern, despite spending the three months in

boots half eaten by dogs. I cherish the time spent in the field with my late, great friend, Mari Pesek, who was the best boat captain in Panama Canal history. I thank Fortuna field manager Carlos Espinosa for always being at the other end of the radio with a pair of jumper cables.

I am indebted to the community and staff of the Smithsonian Tropical Research Institute, especially Ben Turner for sharing his incredible soil datasets and providing intellectual support and encouragement. I thank the staff of Barro Colorado Island for coordinating the logistical nightmare of my fieldwork. I cannot imagine my time in Panama without camaraderie of fellow STRI students and interns, especially KC Cushman and Jenalle Eck.

This project benefited from the excellent research facilities and intellectual community at the University of Illinois. I thank my doctoral committee for their insightful feedback and for the freedom to come and go to Panama as I pleased. I thank Michael Masters and Rudiger Laufhutte for training and technical support with chemical analysis, and I appreciate the hard work of the many students who assisted in the processing of plant samples including Charles Tam, Nathan Stables, Tony Chung, Alex Hathcock, and Iulianna Taritsa.

Finally, I would not be in graduate school with the support of my parents who always encouraged me to ask questions and embrace challenges. I thank my two brilliant and hilarious sisters, Marypat and Emily, for making me luckiest middle child on earth. Most of all, I am deeply grateful for my husband, Mike Gahan, who served as the chief travel agent, cheerleader, and statistical consultant for my dissertation project. I am excited to spend our post-doctoral lives chatting about biodiversity and college football.

To The Green Truck

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CHAPTER 1

INTRODUCTION

BACKGROUND

The tropical climate, characterized by high year round rainfall and temperature, creates ideal growing conditions for trees. Consequently, tropical forests are the most productive and species rich biomass on the planet, harbouring over half of species on Earth (Losos & Leigh, 2004). However, the same conditions that sustain high plant productivity in tropical forests result in strongly weathered soils, depleted in the availability of rock derived elements such as phosphorus (P) (Walker & Syers, 1976). Soil nutrients limits productivity of tropical forests (Vitousek & Sanford, 1986), and influence the distribution of tree species at local (John *et al.*, 2007) and regional scales (Condit *et al.*, 2013).

Despite widespread evidence of limitation by soil nutrients, the strength of this effect and the identity of limiting nutrient(s) are far from homogenous across tropical forests. Nutrient addition experiments have provided examples of tropical forests limited solely by nitrogen N (Vitousek & Farrington, 1997), solely P, neither (Alvarez-Clare *et al.*, 2013), or N and P when applied together (Tanner *et al.*, 1992). A potential explanation for this heterogeneity is variation among tree taxa in nutrient allocation and acquisition strategies (Townsend *et al.*, 2011; Dalling *et al.*, 2015). For example, ectomycorrhizal and arbuscular mycorrhizal associated tropical tree species display contrasting responses to nitrogen addition (Adamek *et al.*, 2009; Homeier *et al.*, 2012), most likely owing to differences in their abilities to acquire N from organic material in the soil. With respect to nutrient allocation, tree species foliar N:P ratios, an indicator of N vs. P

limitation (Koerselman & Meuleman, 1996), vary widely among low and high fertility habitats in tropical forests (Townsend *et al.*, 2007; Dalling *et al.*, 2016), indicating that among species differences in nutrient requirement could generate spatial heterogeneity in nutrient limitation.

While patterns of variation in foliar chemistry have been widely explored across climatic and edaphic gradients (Wright *et al.*, 2004; Fyllas *et al.*, 2009), variation in non-photosynthetic biomass, such as wood, roots, and bark, has receive much less attention. While the concentrations of limiting elements in wood are typically an order of magnitude lower in wood compared to leaves, wood contains the majority of nutrients in tree biomass (Wang *et al.*, 1991; Bond, 2010), and the concentrations of nutrients in wood are a key predictor of rates of carbon turnover from woody biomass at global scales (Weedon *et al.*, 2009; Zanne *et al.*, 2015). This dissertation evaluates how diversity among tree species nutrient allocation strategies influences tropical forest ecosystem and community dynamics, with a particular emphasis on the importance of nutrient allocation to woody biomass.

STUDY SYSTEM

The Isthmus of Panama is an ideal region to study variation in plant-soil interactions. Panama boasts high geological and taxonomic diversity due in large part to its unique biogeographic position at the convergence of Central and South America (Leigh *et al.*, 2014). Furthermore, because the Smithsonian Institution has supported research in Panama since 1923, there is wealth of historical ecological data from numerous scientific installations throughout the country, most notably Barro Colorado Island located in the middle of the Panama Canal. My dissertation takes advantage the work at the Smithsonian Tropical Research Institute (STRI) by evaluating plant soil dynamics in permanent forest plots networks at two sites: the montane forest of Fortuna Forest Reserve in western Panama and the lowland forest of Panama Canal

watershed in central Panama. Lowland and montane tropical forests differ in key characteristics that provide a contrast in nutrient dynamics: montane forests are typically more strongly limited by nitrogen compared to lowland forests (Tanner *et al.*, 1998) in part because lower temperatures at high elevation slows the rate of nitrogen mineralization and fixation. STRI staff scientist Benjamin L. Turner has analyzed the soil chemical characteristics of nearly all permanent forest plots in the Fortuna (Andersen, *et al.*, 2010; Prada *et al. in review*) and Panama Canal (Turner & Engelbrecht, 2011; Condit *et al.*, 2013) networks, finding extreme contrasts in nutrient availability at both sites. In fact, together these plot networks span a range of soil 'available' nutrient concentrations comparable to the range observed throughout the tropics (Gartlan *et al.*, 1986; Baillie *et al.*, 1987; Phillips *et al.*, 2003, Quesada *et al.*, 2009). Furthermore, both sites are associated with long-term nutrient addition experiments, which 1) indicate that both sites are limited by one or more nutrients (Adamek *et al.*, 2009; Wright *et al.*, 2011) and 2) enable us to compare the effect of experimental nutrient limitation to the effect of natural variation in soil nutrient availability.

Fortuna forest reserve: montane forest

The Fortuna plots are located in the Fortuna Forest Reserve (19,500 ha) and the adjacent Palo Seco Forest Protectorate (125,000 ha) in western Panama. This region encompasses old growth, lower montane forest, ranging between 700 and 1500 m asl, with mean annual temperatures varying between 19 and 23°C (Cavelier *et al.*, 1997). There is strong interannual and spatial variability in precipitation among study sites, with annual rainfall ranging from 4000 to 9000 mm per year. A distinct dry season occurs from January to April, but evapotranspiration does not exceed rainfall during this period (Cavelier *et al.*, 1997), with monthly rainfall accumulation exceeding 100 mm per month on average during the dry season in all but one site.

Twelve permanent 1-ha forest plots were established at Fortuna in 2003 in which all trees > 5 cm diameter at breast height (DBH) are mapped, measured, and identified to species. Extensive study of the understory palm community at this site indicates that soil nutrient have the potentially to strongly influence tree species distribution, nutrient allocation, and performance (Andersen *et al.*, 2010, Andersen *et al.*, 2012; Andersen *et al.*, 2014). Prada et al. *in review* found that tree communities are also strongly structured by soil habitat, with only 22% of species shared between soils developed on dacite and rhyolite located < 15 km apart.

Panama Canal watershed: lowland forest

The Panama Canal watershed region consists of semi-deciduous, seasonally moist forest, receiving 2100-2600 mm of annual rainfall and with a mean annual temperature of 27°C (Pyke *et al.*, 2001). In the dry season, dry spells during which evapotranspiration exceeds rainfall for more than 10 days occur at least every other year (Engelbrecht *et al.*, 2006). Successional age of each plot was classified as old growth, mature secondary, or young secondary by Pyke *et al.*, 2001).

In 1980, a 50-ha plot was established on the central plateau of Barro Colorado Island, which is a 1500-ha island isolated by the formation of the Panama Canal. From 1994-2007, a network of 50 1-ha plots were established in the surrounding Panama Canal watershed by the Center for Tropical Forest Science (Pyke *et al.*, 2001; Condit *et al.*, 2013). On BCI and the majority of 1-ha plots, all trees ≥ 1 cm diameter at breast height (1.3 m; DBH) were measured, tagged, and identified to species according to Condit, 1998). Soil phosphorus and dry season rainfall are important predictors of species spatial distribution across the Panama Canal watershed (Condit *et al.*, 2013).

OVERVIEW

In Chapter 2, I evaluate how variation in litterfall along a natural soil fertility gradient compares to the response of litterfall to nitrogen addition at Fortuna Forest Reserve. I compared two years of canopy litterfall collected in five 1-ha plots at Fortuna to litterfall collected for a concurrent nitrogen fertilization experiment. I found that while nitrogen addition increased litterfall slightly (12%), this effect was small compared to the 66% increase in litterfall between forest dominated by canopy palm *Colphothrinax aphanopetala* compared to forest dominated by ectomycorrhizally-associated *Oreomunnea mexicana*, despite both plots being located on the similarly low fertility soils. This study is provides a clear example of how variation in forest functional composition can complicate the relationship between soil nutrient availability and forest productivity. This paper was coauthored by James W. Dalling, Marife Corre, Pedro Cabellero, and a team of undergraduate researchers from the Universidad Autónoma de Chiriquí, who collected litterfall for this project for their undergraduate thesis. The resulting manuscript was published in the journal *Biotropica*.

In Chapter 3, I explore variation among tree species in the allocation of Ca, K, Mg, N, and P to woody biomass along an extreme regional soil fertility gradient. Wood nutrient concentrations varied enormously among species from 4-fold in nitrogen (N) to > 30-fold in calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P). Despite high variation in these concentrations within sites, community-weighted mean wood nutrient concentrations correlated positively with soil Ca, K, Mg, and P concentrations. Substantial variation among species and communities in wood nutrient concentrations suggests that allocation of nutrients to wood, especially P, influences species distributions and nutrient dynamics in tropical forests.

This paper was coauthored by James W. Dalling and Benjamin L. Turner and has been published in *New Phytologist*, where it is available for early view online.

In Chapter 4, I assess whether tree species nutrient allocation and environmental soil nutrient availability influence the resprouting ability of tropical trees. Using long term plot inventory on BCI, I show that multi-stemmed trees are more likely to resprout during their lifetime than single-stemmed trees, and, multiple stem frequency can therefore be used as a proxy of community or species level resprouting tendencies. At the community level, the proportion of woody stems with at least one multiple stem correlated strongly with soil phosphorus availability. These results suggest that soil nutrient availability facilitates resprouting by relieving nutrient limitation to vegetative growth. This process is a novel mechanism by which soil phosphorus availability influences tree recruitment and forest dynamics. This paper was coauthored by James W. Dalling and Benjamin L. Turner and will be submitted to *Journal of Ecology*.

In Chapter 5, I used a sapling defoliation experiment to test if tropical trees remobilize nitrogen and phosphorus stored in stem wood in response to stress. In the four focal sapling taxa examined, wood P reserves were depleted after in response to leaf refoliation where soil P supply was low. And across all saplings, the quantity of phosphorus remobilized from wood correlated with the quantity phosphorus allocated to new leaves. This study is the first to experimentally evaluate the relationship between wood P remobilization and plant growth in tropical trees. This paper was coauthored by James W. Dalling and Benjamin L. Turner and will be submitted to *Ecology*.

In Chapter 6, I summarize the results of my research, and explore directions for future research on this topic. Through evaluation of litterfall at Fortuna and survey of tree species foliar

and woody nutrient content, I demonstrate that the enormous variation in tree species nutrient allocation strategies both within and among soil habitats in tropical forests has important implications for estimating ecosystem services in tropical forests. Perhaps the most novel finding of this work is the finding that while there is taxonomic control over P content of wood, wood P reserves are dynamic, reflecting both soil nutrient supply and P demands of tree growth. Future work evaluating the chemical composition of wood phosphorus stores and the extent to which wood P reserves are remobilized in the presence of other limiting resource such as nitrogen, CO_2, and light could provide insight into how phosphorus limitation will influence the response of tropical forests to global change scenarios.

LITERATURE CITED

Adamek M, Corre MD, Hölscher D. **2009**. Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, panama. *Journal of Tropical Ecology* **25**, 637-647.

Alvarez-Clare S, Mack M, Brooks M. **2013**. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. *Ecology* **94**, 1540-1551.

Andersen KM, Endara MJ, Turner BL, Dalling JW. **2012**. Trait-based community assembly of understory palms along a soil nutrient gradient in a lower montane tropical forest. *Oecologia* **168**, 519-531.

Andersen KM, Turner BL, Dalling JW. **2010**. Soil-based habitat partitioning in understorey palms in lower montane tropical forests. *Journal of Biogeography* **37**, 278-292.

Andersen KM, Turner BL, Dalling JW. **2014**. Seedling performance trade-offs influencing habitat filtering along a soil nutrient gradient in a tropical forest. *Ecology* **95**, 3399-3413.

Baillie IC, Ashton PS, Anderson JAR, Fitzpatrick EA, Tinsley J, others. **1987**. Site characteristics and the distribution of tree species in mixed dipterocarp forest on tertiary sediments in central sarawak, malaysia. *Journal of Tropical Ecology* **3**, 201-220.

Bond WJ. **2010**. Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant and Soil* **334**, 47-60.

Cavelier J, Jaramillo M, Solis D, de León D. **1997**. Water balance and nutrient inputs in bulk precipitation in tropical montane cloud forest in panama. *Journal of Hydrology* **193**, 83-96.

Condit R. 1998. *Tropical forest census plots: Methods and results from barro colorado island, panama and a comparison with other plots*: Springer Science & Business Media.

Condit R, Engelbrecht BMJ, Pino D, Pérez R, Turner BL. **2013**. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences* **110**, 5064-5068.

Dalling JW, Heineman K, González G, Ostertag R. Geographic, environmental and biotic sources of variation in the nutrient relations of tropical montane forests.

Dalling JW, Heineman K, Lopez OR, Wright SJ, Turner BL. 2016. Nutrient availability in tropical rain forests: The paradigm of phosphorus limitation *Tropical tree physiology* (pp. 261-273): Springer.

Engelbrecht BMJ, Dalling JW, Pearson TRH, Wolf RL, Galvez DA, Koehler T, Tyree MT, Kursar TA. **2006**. Short dry spells in the wet season increase mortality of tropical pioneer seedlings. *Oecologia* **148**, 258-269.

Fyllas NM, Patino S, Baker TR, Bielefeld Nardoto G, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna V, Mercado LM et al. 2009. Basin-wide variations in foliar properties of amazonian forest: Phylogeny, soils and climate. *Biogeosciences* **6**, 2677-2708.

Gartlan JS, Newbery DM, Thomas DW, Waterman PG. **1986**. The influence of topography and soil phosphorus on the vegetation of korup forest reserve, cameroun. *Vegetatio* **65**, 131-148.

Homeier J, Hertel D, Camenzind T, Cumbicus NL, Maraun M, Martinson GO, Poma LN, Rillig MC, Sandmann D, Scheu S. **2012**. Tropical andean forests are highly susceptible to nutrient inputs—rapid effects of experimental n and p addition to an ecuadorian montane forest. *PLoS One* **7**, e47128.

John R, Dalling JW, Harms KE, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M et al. 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 864-869.

Koerselman W, Meuleman AFM. **1996**. The vegetation n:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* **33**, 1441-1450.

Leigh EG, O'Dea A, Vermeij GJ. **2014**. Historical biogeography of the isthmus of panama. *Biological Reviews* **89**, 148-172.

Losos EC, Leigh EG. 2004. *Tropical forest diversity and dynamism: Findings from a large-scale plot network*: University of Chicago Press.

Phillips OL, Vargas PN, Monteagudo AL, Cruz AP, Zans M-EC, Sánchez WG, Yli-Halla M, Rose S. **2003**. Habitat association among amazonian tree species: A landscape-scale approach. *Journal of Ecology* **91**, 757-775.

Pyke CR, Condit R, Aguilar S, Lao S. **2001**. Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science* **12**, 553-566.

Quesada CA, Lloyd J, Schwarz M, Baker TR, Phillips OL, Patiño S, Czimczik C, Hodnett MG, Herrera R, Arneth A et al. 2009. Regional and large-scale patterns in amazon forest structure and function are mediated by variations in soil physical and chemical properties. *Biogeosciences Discussions* **6**, 3993-4057.

Tanner E, Kapos V, Franco W. **1992**. Nitorgen and phosphorus fertilization effects on venezuelan montane forest trunk growth and litterfall. *Ecology* **73**, 78-86.

Tanner EVJ, Vitousek PM, Cuevas E. **1998**. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**, 10-22.

Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. **2007**. Controls over foliar n:P ratios in tropical rain forests. *Ecology* **88**, 107-118.

Townsend AR, Cleveland CC, Houlton BZ, Alden CB, White JW. **2011**. Multi-element regulation of the tropical forest carbon cycle. *Frontiers in Ecology and the Environment* **9**, 9-17.

Turner BL, Engelbrecht BM. **2011**. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**, 297-315.

Vitousek PM, Farrington H. **1997**. Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry* **37**, 63-75.

Vitousek PM, Sanford RL. **1986**. Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics*, 137-167.

Walker T, Syers JK. **1976**. The fate of phosphorus during pedogenesis. *Geoderma* **15**, 1-19.

Wang D, Bormann FH, Lugo AE, Bowden RD. **1991**. Comparison of nutrient-use efficiency and biomass production in five tropical tree taxa. *Forest Ecology and Management* **46**, 1-21.

Weedon JT, Cornwell WK, Cornelissen JHC, Zanne AE, Wirth C, Coomes DA. **2009**. Global meta-analysis of wood decomposition rates: A role for trait variation among tree species? *Ecology Letters* **12**, 45-56.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M et al. 2004. The worldwide leaf economics spectrum. *Nature* **428**, 821-827.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE et al. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616-1625.

Zanne AE, Oberle B, Dunham KM, Milo AM, Walton ML, Young DF. **2015**. A deteriorating state of affairs: How endogenous and exogenous factors determine plant decay rates. *Journal of Ecology* **103**, 1421-1431.

CHAPTER 2[1]

VARIATION IN CANOPY LITTERFALL ALONG A PRECIPITATION AND SOIL FERTILITY GRADIENT IN A PANAMANIAN LOWER MONTANE FOREST

ABSTRACT

Fertilization experiments in tropical forests have shown that litterfall increases in response to the addition of one or more soil nutrients. However, the relationship between soil nutrient availability and litterfall is poorly defined along natural soil fertility gradients, especially in tropical montane forests. Here we measured litterfall for two years in five lower montane 1-ha plots spanning a soil fertility and precipitation gradient in lower montane forest at Fortuna, Panama. Litterfall was also measured in a concurrent nitrogen fertilization experiment at one site. Repeated-measures ANOVA was used to test for site (or treatment), year, and season effects on vegetative, reproductive, and total litterfall. We predicted that total litterfall, and the ratio of reproductive to leaf litterfall, would increase with nutrient availability along the fertility gradient, and in response to nitrogen addition. We found that total annual litterfall varied substantially among 1-ha plots (4.78 Mg ha^{-1} yr^{-1} to 7.96 Mg ha^{-1} yr^{-1}), and all but the most aseasonal plot showed significant seasonality in litterfall. However, litterfall accumulation did not track soil nutrient availability; instead forest growing on relatively infertile soil, but dominated by an ectomycorrhizal tree species, had the highest total litterfall accumulation. In the fertilization plots, significantly more total litter fell in nitrogen addition relative to control plots, but this increase in response to nitrogen (13%) was small compared to variation observed among 1-ha

[1] **Heineman, K. D.**, *et al.,* 2015 Variation in canopy litterfall along a precipitation and soil fertility gradient in a Panamanian lower montane forest. Biotropica, 47, 300-309.

plots (66%). These results suggest that while litterfall at Fortuna is nutrient-limited, compositional and functional turnover along the fertility gradient obscure any direct relationship between soil resource availability and canopy productivity.

INTRODUCTION

Litterfall is the deposition of leaves, twigs, reproductive tissue, and other organic matter from the forest canopy onto the forest floor. Litterfall represents a large fraction (~30%) of forest net primary productivity (NPP; Aragão *et al.*, 2009) with important impacts on soil microbial communities and soil carbon storage (Sayer *et al.*, 2012). Litter production has also been used as an indicator of nutrient limitation to forest productivity. In tropical forests, total litter biomass has been shown to increase relative to unfertilized controls in response to experimental N addition (Adamek *et al.*, 2009), P addition (Wright *et al.*, 2011), or both (Tanner *et al.*, 1992).

While experimental nutrient addition suggests that litterfall is sensitive to soil nutrient availability, comparisons of litterfall rates across natural productivity gradients show contrasting effects on canopy net primary productivity. In a comparison across 81 sites in South American tropical forests, Chave *et al.*, (2010) found that annual rainfall did not explain any variation in annual litterfall. Furthermore, litterfall did not vary consistently with soil type, except for reduced litterfall on the most infertile white sand soils. In a similar comparison across ten lowland Amazonian sites ranging from infertile white sand forest to fertile Terra Preta forest, Aragão *et al.* (2009) found that litterfall did increase significantly with soil P availability (see also Vitousek, 1984, Silver, 1994).

Not only does litterfall represent an important allocation of forest productivity, it also represents the primary means by which labile nutrients are returned to the soil and made available for plant nutrition. The production of low quality, difficult to decompose litter with

high carbon to nutrient ratios is simultaneously an adaptation to a low nutrient environment and a perpetuator of nutrient limitation. In particular, the concentration of nitrogen in litterfall varies widely among forest types and correlates with the amount of nitrogen deposited by litterfall each year (Vitousek 1984). Recent studies have suggested that forests differing in the relative abundance of taxa with ectomycorrhizal (EM) vs. arbuscular mycorrhizal (AM) fungal associations may have marked differences in nitrogen cycling related to differences in litter quality and decomposition in AM and EM stands (Phillips *et al.*, 2013). Lower montane neotropical forests represent an ecotone where lowland AM-associated communities transition to montane communities dominated by EM associated members of the Fagales and, therefore, present an opportunity to explore differences in litterfall patterns between tree communities with contrasting functional groups that are related to nutrient acquisition.

Despite the large number of litter collection studies, canopy productivity remains relatively poorly estimated for montane forests relative to lowland tropical forest. The Chave *et al.* (2010) meta-analysis of 81 South American forests contained data from only five montane plots located in two study sites (Veneklaas, 1991, Röderstein *et al.*, 2005). Another pan-tropical compilation of litterfall datasets (Leigh, 1999) includes more montane sites (14 of 52 sites have > 800 m elevation; replicate plots and years excluded); however interpretation of differences in litterfall rates among these more diverse sites is potentially confounded by differences in litterfall collection methods and litter classification (Proctor, 1983).

Litterfall rates might be expected to be low in montane forests relative to lowland forests because total NPP generally declines with elevation (Raich *et al.*, 1997, Kitayama & Aiba, 2002, Moser *et al.*, 2011). For example, Girardin *et al.*, (2010) found that no sites > 1000 m asl had higher NPP than any lowland Amazonian site, including sites located in infertile white sand

forests. Furthermore, canopy NPP is positively correlated with stem NPP across lowland and montane sites (Aragão *et al.* 2009), and also declined with elevation in the Girardin *et al.* (2010) study. Constraints on productivity resulting from reduced irradiance or nutrient availability in montane forest might also be expected to impact reproductive allocation. Although data on fruit production are more limited than leaf or total litterfall, production of reproductive organs has been shown to decline strongly with elevation, and as a proportion of total canopy NPP from 1000-3000 m elevation (Table 2 in Moser *et al.,* 2011). Comparisons across soil gradients also suggest that proportional reproductive allocation increases with increasing fertility (Van Schaik & Mirmanto, 1985; Chave *et al.*, 2010).

In this study we compare total, vegetative, and reproductive litterfall rates across five one-hectare plots in lower montane forest in western Panama with contrasting soil fertility and precipitation regimes, and across a replicated nitrogen addition experiment at one site. We hypothesized that if soil nutrient availability is a strong driver of forest productivity in montane forest, and if leaf production is prioritized over investment in reproduction under nutrient poor conditions, then total litter biomass, and the ratio of reproductive to vegetative biomass will increase with soil fertility, and with nitrogen addition. In contrast, we predicted that in these wet forests, rainfall regime would have a stronger effect on seasonality of litterfall rather than on total litterfall production.

MATERIALS AND METHODS

Study site

Fieldwork was conducted at the Fortuna Forest Reserve (19,500 ha), located in western Panama in the province of Chiriqui, and the adjacent Palo Seco Forest Reserve (125,000 ha) in the province of Bocas Del Toro (Fig. 2.1). The area encompasses lower montane forests ranging

between 700 and 1500 m asl. Mean annual rainfall at the study sites ranges between ~5100 and 7200 mm depending on orographic position (Table 2.1). There is seasonality in rainfall, but mean monthly rainfall in the drier months (January to April) exceeds 100 mm at all the sites. Mean annual temperature ranges from 19 to 22ºC across the study sites (Andersen *et al.*, 2010).

Within the study region, litterfall was measured within five one-hectare permanent forest inventory plots (Fig. S1). The plots differ in canopy tree species composition (Prada et al., *in review*), reflecting underlying geology and soils (Table 1). Two plots occur on low fertility rhyolitic tuff (Honda and Chorro), and support forests dominated by the ectomycorrhizal tree *Oreomunnea mexicana* and the canopy palm *Colpothrinax aphenopetala*, respectively. Two plots occur on intermediate fertility andesite, and support high diversity mixed forest (Samudio and Palo Seco), and one plot occurs on high fertility porphyritic dacite, supporting mixed Lauraceae and oak forest (Hornito).

A detailed inventory of soil samples in each 1-ha plot was conducted in 2008 to evaluate the influence of soil variables in structuring understory palm communities. Soil samples were taken from 0 to 10 cm depth from 13 locations per plot and analysed in the STRI soil analysis lab. Soil properties measured included extractable inorganic nitrogen (NH_4 and NO_3), exchangeable phosphorus and cations (Al, Ca, Fe, K, Mg, Zn) concentrations (Mehlich-3 extraction), pH, bulk density, net nitrogen mineralization and nitrification rates. We list a subset of these measures in Table 2.1. For detailed methods of each soil property measured, see Andersen et al. (2010).

Study plots and sampling regime

Thirteen 0.71 m^2 litter traps were equally spaced across each 1-ha plot in a stratified random design. Traps were constructed out of polyvinyl chloride tubing, raised 1 m above the

ground with 2 mm nylon mesh suspended to capture falling litter. Litter was collected from each trap every two weeks from August 2008-December 2010 and sorted into six categories: leaf, bark, branch, fruit, flowers, and other (principally epiphytes and canopy soil). Litter samples were dried at 60 ^{o}C for 72 hours and weighed. Based on repeated weighing of litter samples after 72, 96, and 120 hours for the first four collection periods, we determined that 72 hours of drying was sufficient for samples to achieve constant mass. In addition, on three sampling dates between November 2008 and July 2009 random subsamples of leaf litter were collected from each site, pooled within a site, ground and analysed for total carbon and nitrogen using a Thermo Flash EA112 analyzer (CE Elantech, New Jersey, USA).

In addition to litterfall data collected across the five plots representing a natural gradient in soil fertility, we also included data on litterfall collected using the same methodology in four 0.16-ha paired control and nitrogen-addition plots, which are part of an on-going nitrogen manipulation experiment 'NITROF' ~150 m from the Honda plot (Adamek *et al.,* 2009). NITROF plots were established in 2006. Fertilized plots received 125 kg urea-N/ha/yr, applied four times per year. Litterfall was collected every two weeks from four 0.5 m^2 traps per plot. Traps were constructed in the same way as those used in the 1 ha plots and litter was sorted, dried and weighed using the same criteria.

Previous authors have suggested that traditional measures of canopy productivity underestimate the litterfall contribution of palm fronds that are too large to fit into standard litterfall baskets (Chave *et al.,* 2010). A comparison of palm litterfall accumulation in three Brazilian forest plots found that 0.32 m^2 litter fall baskets collected 18-30 times less palm litter biomass per unit area than 5 x 5 m ground plots (Villela & Proctor, 1999). To estimate leaf litterfall produced by canopy palms at Chorro, we measured leaf production in the canopy palm

Colphothrinax aphanopetala for one year (August 2011- July 2012) in 30 palms located inside or within 100 meters of the Chorro 1-ha plot. We selected individuals with > 10 leaves for which all fully expanded leaves could be tagged from the ground (average height of 4.82 m). We assumed that leaf production of subcanopy palms would be similar to leaf production of taller canopy palms due the high light availability in the understory of this forest (8-10%), and because the shorter palms measured and adult canopy palms did not differ significantly in their average number of leaves.

In July 2012, untagged leaves were counted to determine annual leaf production. We measured the dry mass of three senescing leaves on one *Colpothrinax* individual harvested for destructive biomass estimation. To estimate annual leaf litterfall biomass for *Colpothrinax* in Chorro, we multiplied senescing leaf dry mass (1.365 kg $leaf^{-1}$) × average annual leaf production (2.6 leaves yr^{-1}) × number of individuals *Colpothrinax* recorded in the 2008 census of the Chorro plot (173 trees).

Data analysis

Repeated-measures ANOVA models were used to test for a plot × year interaction on annual accumulation of total, leaf, reproductive, and the ratio of reproductive to leaf litterfall in the five 1-ha plots in 2009 and 2010. Because there is considerable spatial variation in canopy litterfall (Burghouts *et al.*, 1998), we used litterfall basket as unit of replication to quantify uncertainty in our estimates of annual litterfall. This approach is consistent with how of other studies comparing litterfall across forest types have been performed (e.g., Proctor, 1983; Dantas & Phillipson, 1989; Dezzeo & Chacón, 2006).

We tested for seasonality in the monthly accumulation rate of each litterfall component in the 1-ha plots using models including a plot × season interaction, where dry season was defined

as 1 January – 30 April of each year. We calculated the annual or seasonal accumulation of litter in each basket by summing the dry weight of each litter component across biweekly collection periods for the specified time interval. In each model, litterfall basket was designated as a random effect to reduce the number of degrees of freedom incurred by the repeated measurement of the same locations each year or season. Denominator degrees of freedom used to determine P values of fixed effects were calculated using the lmerTest package (Kuznetsova *et al.,* 2014), which applies the Satterthwaite approximation (Satterthwaite, 1946) to linear mixed effects models created in the *lme4* package (Bates *et al.,* 2014) in R (R Core Development Team, 2014).

We tested for the effects of nitrogen addition and year on annual accumulation of the total, leaf, and reproductive litterfall in the NITROF plots for the same time period (2009-2010) using a two-way ANOVA. We do not present the results for the treatment × year interaction, as it was not significant in any comparison. Each 40 × 40 m plot, including four control and four nitrogen-addition plots, was treated as an independent unit of replication.

RESULTS

Among site variation in litterfall

For total annual litterfall, there were significant differences among the 1 ha plots at Fortuna (Fig. 2.1a), with mean dry mass ranging from 4.78 Mg ha^{-1} yr^{-1} to 7.96 Mg ha^{-1} yr^{-1} (F = 9.16, df = 4,60, P < 0.001), and in leaf litterfall (range 3.00 Mg ha^{-1} yr^{-1} to 5.16 Mg ha^{-1} yr^{-1}; F = 14.96, df = 4,60, P < 0.001). Elemental analysis of litter samples revealed significant variation in leaf litter nitrogen concentration (F = 6.31, df = 4,11, P = 0.070), and C:N (F = 5.92, df = 4,11, P = 0.008). Leaf litter N was lowest on the two rhyolite soils (Chorro and Honda) and highest at Palo Seco and Hornito; variation in N was also reflected in litter C:N (Table 1). Differences in total litterfall and litter chemistry among sites also resulted in significant differences in the

amount of nitrogen deposited by litterfall, varying two-fold among plots from 56 kg N ha^{-1} yr^{-1} to 120 kg N ha^{-1} yr^{-1} ($F = 16.19$, df = 4,60, $P < 0.001$).

Support for our hypothesis that litterfall would correlate with soil nutrient availability was weak. While total litterfall and leaf litterfall tended to increase with soil fertility (Fig. 2.2a), the second most infertile site, Honda, had the highest litterfall. Similarly, although the total amount of N deposited by litter was highest in the most fertile site, Hornito, and lowest in the least fertile site, Chorro, in both 2009 and 2010, nitrogen in litterfall did not correspond directly with soil fertility due to the relatively high N production in Honda (Figure 2.3a). Variation in litterfall among sites could be explained in part by variation in basal area among sites (Table 2.2). When total litterfall was expressed on a per unit basal area basis, Honda still had the highest litter production rate during the two year collection period (Fig. 2.2b), but overall variance in total litterfall explained by the site was smaller when litterfall was adjusted by basal area (site effect per land area: $F = 9.16$; plot effect per basal area: $F = 5.37$; Table S1).

The accumulation of total reproductive litterfall differed significantly among sites ($F = 2.90$, df = 4,60, $P = 0.029$), as did the ratio of reproductive to leaf litterfall ($F = 5.80$, df = 4,60, $P < 0.001$). However, contrary to our prediction, the ratio of reproductive to non-reproductive litter was *highest* at the lowest fertility site, Chorro (Fig. 2.3b). High proportional allocation to reproduction at Chorro reflected both high reproductive biomass and low leaf and support (i.e., branches, bark, epiphytes) biomass (Fig. 2.2).

Interannual variation in litterfall

The magnitude of interannual variation in total litterfall was highly site specific (range in percent change: 2 – 29%). Significantly more litter fell in 2009 (the year of a large storm event) than in 2010 in two sites: Honda and Samudio, while total accumulation in Palo Seco, the only

site on the Caribbean slope, was greater in 2010 than 2009 (Fig. 2.2; site × year interaction: F = 4.30, df = 4,60, P = 0.004). Across all plots, significantly more leaf litter fell in 2009 than in 2010; however the magnitude of this difference was small relative to interannual variation in total litterfall (range in percent change 10-15%; year effect: F = 4.06, df = 1,60, P = 0.048). There was a significant site × year interaction in the production of reproductive litterfall: the accumulation of fruits and flowers differed substantially between 2009 and 2010 at Honda and Hornito (Fig 2.3b), while reproductive litterfall did not differ between years at Palo Seco, Chorro or Samudio (range 0.1 – 71%; site × year interaction F = 3.28, df = 4,60, P = 0.017).

Seasonality

Two large peaks in litterfall were observed over the study period in all the five sites (Fig. 2.4), occurring in the early dry season (February 2009 and January 2010). These peaks coincided with severe storms and accounted for up to 40 percent of annual litterfall at Samudio in 2009, and 21 percent of annual litterfall at Samudio in 2010. When total litterfall was calculated for the dry and wet seasons there was a significant site × season interaction in total litterfall production (F = 3.06, df = 4,190, P < 0.001). With the exception of the aseasonal site of Palo Seco, there was significantly more monthly total, leaf, and support litterfall in the dry season than wet season (Fig. 2.5a,b,d). In contrast, monthly accumulation of reproductive litterfall was greater in the wet season than the dry season (season effect: F = 5.31, df = 1,190, P = 0.020; Fig. 2.5c)

Response to nitrogen addition

Over the same period (2009 and 2010), total litterfall ranged from 6.04 $\pm$ 0.15 Mg/ha/yr to 7.95 $\pm$ 1.66 Mg/ha/yr (mean dry mass $\pm$ standard deviation) across the eight NITROF plots (Fig. 2.6). Total litterfall rates at the NITROF plots were therefore comparable to those measured at the nearby site of Honda (7.96 $\pm$ 1.04 Mg/ha/yr). N fertilization resulted in a significant (13%)

increase in total litterfall (Fig. 5, $F = 5.90$, df = 1,13, $P = 0.029$), and 11 percent increase in leaf litterfall relative to unfertilized controls, which was not statistically significant ($F = 3.08$, df = 1,13, $P = 0.10$). Neither reproductive litterfall ($F = 0.06$, df= 1,13, $P = 0.80$) nor the ratio of reproductive to leaf litterfall ($F = 0.41$, df = 1,13, $P = 0.53$) differed significantly between treatments. No component of litterfall accumulation differed between 2009 and 2010 (Table 2.3).

Colpothrinax leaf production

The annual leaf litter accumulation of *Colpothrinax* was estimated as 0.61 Mg ha^{-1} yr^{-1} when calculated as a product of the 2012 leaf production rate and species abundance in Chorro plot. Therefore, if litterfall traps failed to collect any *Colpothrinax* leaves, leaf litterfall in Chorro, measured as 3.00 Mg ha^{-1} yr^{-1} in litterfall traps, would theoretically be underestimated by 16 percent, and total litterfall underestimated by 11 percent. While *Colpothrinax* made up 30 percent of the basal area in Chorro, it contributed at most 20 percent to overall leaf litterfall.

DISCUSSION

Litterfall rates in a lower montane forest

Although total litterfall accumulation ranged widely among permanent plots measured along the Fortuna soil gradient (4.78 Mg ha^{-1} yr^{-1} to 7.96 Mg ha^{-1} yr^{-1}), this variation did not correspond directly with differences soil nutrient availability among sites. At Fortuna, the lowest and highest litterfall rates were recorded at the two sites with the lowest nitrification rate, Mehlich-extractable P and soil pH, and with the lowest foliar nitrogen concentrations: Chorro and Honda. The Chorro site, with the lowest litterfall, has developed on a two-meter deep layer of coarse white silica-rich rhylotic tuff, with a shallow organic horizon < 10 cm deep (Table 2.1; Anderson *et al.,* 2009). Although floristically distinct, the Chorro soils are analogous to

Amazonian white sand forests and its productivity appears to be comparable; total litterfall at Chorro was within the 95% confidence intervals of six white-sand forests reported by Chave *et al.* (2010). Soils at the Honda site, < 1 km from Chorro, are also derived from rhyolite, but are better developed (Table 2.1) with mineral soil extending 60 cm below the surface. Given the low fertility of the Honda site, its high litterfall compared to forests developed on andesite and dacite likely reflects a compositional difference. A larger fraction of the basal area of Honda consists of ectomycorrhizal (EM) tree species (*Oreomunnea* and *Quercus*) than the other sites (Table 2.1). EM species are thought to have an advantage over arbuscular mycorrhizal (AM) tree species where nitrogen is limiting, as EM fungi are capable of accessing sources of organic nitrogen unavailable to AM fungi (Hodge *et al.*, 2001; Read & Perez-Moreno, 2003).

The range of annual litterfall we observed (6.37 ± 1.25) falls below the mean litterfall rate of 8.61 ± 1.91 Mg ha^{-1} yr^{-1} recorded for 81 mostly lowland sites (Chave *et al.,* 2010), but is comparable to five montane sites ranging from 1890 - 3370 m elevation (7.06 ± 3.72 Mg/ha/yr) included in the same paper and 13 montane sites 1000 – 2500 m elevation listed in Vitousek (1984) (7.43 ± 2.29 Mg/ha/yr). Whereas the Fortuna sites had slightly lower total litter accumulation on average than previously measured montane sites, the 1-ha plots measured here had greater total litter N production (ranging from 56 - 124 kg ha^{-1} yr^{-1}) compared to the Vitousek (1984) sites, which ranged from 31 - 90 kg ha^{-1} yr^{-1}. This difference between our data and previous results was driven by low dry mass:N ratios (high litter N concentrations), ranging from 55-84 at Fortuna compared to 80-180 in Vitousek (1984), indicating that the Fortuna sites as a whole have lower within-stand nitrogen use efficiency. In contrast to Vitousek (1984), dry mass:N ratio did not correlate with total N production for the five Fortuna plots, due in part to high productivity, but poor litter quality at the low fertility, EM-dominated Honda site.

The increased forest nitrogen use efficiency of EM-dominated Honda compared to the other AM-dominated sites at Fortuna is consistent with the mycorrhizal-associated nutrient economy (MANE) hypothesis outlined by Philips *et al.* (2013). This hypothesis states that forests containing AM-associated species produce high quality litter with a relatively fast decomposition rate because nutrient uptake by AM fungi relies on scavenging for nutrients mineralized by saprophytic fungi. In contrast, EM fungi, while more costly in terms of plant carbon investment, are capable of metabolizing nitrogen from organic material directly (Chalot & Brun, 1998), and are therefore advantageous relative to AM-fungi where N is limiting to plant growth. If EM-associated stands produce nutrient poor, slowly decomposing litter, then these stands should retain a larger proportion of mineralized inorganic N within the ecosystem. Consistent with this pattern, the EM-dominated site, Honda, returned a relatively large amount of N to the ground via recalcitrant litterfall with high C:N ratios. Differences in nutrient cycling pathways among AM, EM, and mixed forest stands constitute one avenue by which compositional change may complicate the relationship between nutrient availability and canopy productivity and should be further evaluated in lower montane forests where both associations are abundant.

Analyses of canopy productivity however should keep in mind that litterfall is not a direct measure of the production of leaves, reproductive tissues, wood, bark, and epiphytes. Plant material consumed by herbivores or decomposed in the canopy does not fall into litter baskets and, therefore, could result in underestimation in canopy NPP estimates (Clark *et al.*, 2001). Furthermore, the loss of plant material from the canopy is not necessarily synchronous with production of new biomass during the year it is measured, especially in the case of coarse woody structural material (Chambers *et al.*, 2001). However, given that the aim of this study is compare litter production among sites and treatments rather than construct quantitatively precise carbon

budgets, the strength of these sources of uncertainty would have to be highly variable across space to qualitatively influence our results.

Litterfall response to nitrogen fertilization

This study provided an opportunity to evaluate the magnitude of nitrogen-limitation to canopy litterfall in a mixed EM-AM community through an on-going nitrogen fertilization (NITROF) experiment alongside the Honda plot. N fertilization effects on litterfall were assessed for the same two-year period as our other five sites, and were measured three years after fertilization treatments were initiated. While a previous N fertilization experiment in a montane forest in Venezuela did not observe significant effects on litterfall until the fourth year after first fertilization (Tanner *et al.,* 1992), at Fortuna fertilization effects were observed in the first two years of fertilization (2006-7), with on average 11 percent higher total litterfall and 17 percent higher leaf litterfall in the nitrogen addition treatment relative to control (Adamek *et al.,* 2009). In 2009-10 total litterfall was 13 percent higher in the treatment plots, while leaf litter mass was 11 percent above controls. These differences after heavy nitrogen addition remain relatively small compared to landscape-scale variation among our other sites (Fig. 2). Excluding Chorro, total litterfall and leaf litterfall at Honda was 31 percent and 38 percent higher, respectively, than the nearby site of Samudio (Fig. 5). These results suggest that compositional differences, or nutrient limitation by elements other than nitrogen, notably phosphorus, likely account for variation in productivity at Fortuna.

Further evidence of P limitation at Fortuna can be seen in the foliar chemistry of the plant community: 77 of 91 species sampled across the Fortuna soil gradient had foliar N:P ratios > 16 (Dalling *et al.*, 2015), which is the empirically derived threshold for discerning plant P limitation (Koerselman & Meuleman, 1996). P has been found to be co-limit litterfall production in

fertilization experiments in tropical montane forests in Venezuela (Tanner *et al.,* 1992) and Ecuador (Homeier *et al.*, 2012). However, P availability alone cannot explain the difference in litterfall among sites, given that Hornito and Honda vary 10 fold in Mehlich P, but do not differ significantly in total litterfall accumulation.

Seasonal and annual variation in litterfall

We predicted that while annual litterfall would respond to soil fertility, the seasonality of litterfall would be associated with rainfall regime. Although the five sites are separated by a linear distance of < 14 km, the proportion of annual rainfall that occurs during the three-month dry season ranges between 15 and 29 percent (Table 2). Large differences in the seasonality of total and leaf litterfall were observed among sites. With the exception of the most aseasonal site, Palo Seco, significantly more litter fell per month in the dry season than in the wet season, and, in the three most seasonal sites, the majority of annual leaf litter fell in the four-month dry season. These results are therefore consistent with a more general, but weak relationship between litter and rainfall seasonality for tropical South America (Chave *et al.,* 2010), though that study noted that litter seasonality was weakest for montane sites.

Most litterfall datasets for tropical forests are based on a single year of sampling. For lowland tropical forest this appears to provide an adequate estimation of litter production, with interannual variation < 10 percent of mean litterfall (Chave *et al.* 2010). However, in this study, interannual variation in total litterfall was higher than 10 percent for three of the five sites, with a maximum difference of 30 percent between 2009 and 2010. Yearly leaf litterfall accumulation was more constrained, as no site differed more than 15 percent between years. Reproductive tissues showed extremely high variation (71%) between years in Honda, the site dominated by *Oreomunnea mexicana*, supporting the notion that masting is an important component of the

success of monodominant ectomycorrhizal forests (e.g., Green & Newbery, 2002; Henkel *et al.*, 2005).

However, because reproductive litter only accounted for an average of 8 percent of annual litterfall across sites, interannual differences in total litterfall were primarily driven by fallen support tissue (branches, twigs) and epiphytes during severe wind disturbance events. In both 2009 and 2010, a large fraction of litterfall occurred in one 2-week census period associated with high dry season winds. Therefore, more than one year of litterfall collection may be necessary to quantify litterfall accumulation in montane forests where wind disturbance plays a larger role in forest dynamics than in lowland forest.

Variation in components of canopy litterfall

In addition influencing the annual accumulation and seasonality of litterfall, we also expected the variability among sites in precipitation and fertility to influence the relative proportion of canopy productivity allocated to leaves, structural support, reproductive organs, and epiphytes. Epiphytes can account for up to 40 percent of total litterfall in montane forest (Hölscher *et al.*, 2004), with abundance increasing with forest age and rainfall (Wolf, 1993, Koehler *et al.*, 2009). At Fortuna, the litterfall fraction consisting of epiphytes and canopy soil was remarkably consistent (range 18-25%) across sites with varying total rainfall. Differences in epiphyte production therefore do not account for differences in litterfall rates among sites. One other possibility is that variation in litter production among sites simply reflects differences in aboveground biomass rather than differences in productivity. While differences among sites in basal area are substantial, litterfall per unit basal area remained significantly higher at Honda than at remaining sites except Palo Seco in 2010.

Given the potentially confounding effect of epiphyte biomass, leaf litter production may be a better metric for comparison among tropical forests (Röderstein *et al.* 2005). At Fortuna, rank order in annual leaf litterfall accumulation among sites closely paralleled rank order in total litterfall accumulation (Fig. 2). Overall, mean leaf litterfall at the five Fortuna sites (4.04 $\pm$ 0.91 Mg ha^{-1} yr^{-1}) was comparable to values for 19 other montane sites reported in Röderstein *et al.* (2005) (4.87 $\pm$ 1.55 Mg/ha/yr), and with a subset of seven lower montane forests ranging from 1000-1550 m elevation (4.82 $\pm$ 1.12 Mg ha^{-1} yr^{-1}). The exception in this study of significantly lower leaf litter at Chorro (3.00 Mg ha^{-1} yr^{-1}), when compared to the other Fortuna sites, may be a consequence of the dominance of canopy palms. At Chorro three palm species, *Wettinia quinaria*, *Euterpe precatoria*, and *Colpothrinax aphanopetala* accounted for 42 percent of the basal area of the plot. Adequately sampling leaf litter production in palm-dominated forest is a recognized challenge (Chave *et al.,* 2010), as intact falling palm fronds are unlikely to be captured in our traps. Calculation of *Colpothrinax* leaf litterfall, based on a one-year leaf production census, revealed that if no *Colpothrinax* frond fell in our litter baskets, leaf litter production would have been underestimated by 0.61 Mg ha^{-1} yr^{-1}. However, even with a 0.61 Mg ha^{-1} yr^{-1} increase in canopy litterfall, Chorro would remain the least productive of our study sites. If the leaf production rate represents a reliable estimate of litterfall rates for palms, then the potential contribution of *Colpothrinax* to canopy productivity appears to be quite small. While *Colpothrinax* accounts for 30 percent of basal area in the Chorro plot, its litterfall accounts for up to 16 percent of leaf litter (if no palm fronds were captured in baskets) or 20 percent (if baskets accurately sampled palm litterfall). Slow leaf turnover may be an important functional trait allowing canopy palms to reach high abundance relative to woody dicots on this extremely

infertile rhyolitic soil because increased leaf longevity provides a key mechanism by which plants increase nutrient use efficiency (Escudero *et al.*, 1992, Cordell *et al.*, 2001).

Litterfall as a metric of reproductive resource allocation

We predicted that proportional investment in flowers and fruits would increase with soil fertility if nutrients are preferentially allocated to leaves over support tissues in low fertility sites (van Schaik & Mirmanto, 1985, Chave *et al.,* 2010). In this study, we observed significantly *higher* allocation to reproduction at Chorro than the remaining sites. However this may be the result of either underestimating leaf litter production, or overestimating reproductive production if the dominant palm species at this site engage in mast fruiting. More generally, Chave *et al.* (2010) have argued that lower investment in reproduction occurs in low phosphorus eastern Amazonian forests, and that mast fruiting may be more frequent in low fertility sites (see also Ichie & Nakagawa, 2013). Low-diversity forest stands, or stands where a few species attain high relative abundance may require many years of litterfall collection to accurately characterize reproductive investment.

The NITROF experiment also provides an additional opportunity to examine whether nitrogen addition impacts reproductive resource allocation. In 2006-7 there was on average 31 percent higher reproductive litterfall in the nitrogen addition than control treatments (Adamek *et al.,* 2009); however by 2009-10 reproductive litterfall was 6 percent lower in the nitrogen addition treatment. Since reproductive tissues tend to have high concentrations of nitrogen and other potentially limiting elements relative to vegetative tissue (Kuo *et al.*, 1982, Grubb *et al.*, 1998), declining reproductive resource allocation in the N addition plots might represent progressive P or K limitation. Consistent with this result, foliar P concentrations have significantly decreased in the nitrogen addition relative to control plots from 2006-2013, while

foliar N concentrations have remained unchanged in both treatments (A. Hathcock, unpubl. data).

In conclusion, our results provide additional support for reduced litterfall in mid-elevational forests, but in contrast to earlier studies highlight the importance of forest composition, rather than soil fertility, as a determinant of canopy productivity. Although relatively short-term measurements of litterfall may be adequate to characterize leaf litter production rates, measurement of reproductive litterfall may require longer sampling periods to account for mast flowering and fruiting, especially in forests where one tree species achieves high relative abundance.

ACKNOWLEDGMENTS

We thank the Government of Panama's Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) for providing financial support for this project through the international research collaboration grant COLO08. The NITROF experiment was funded by the Robert Bosch Foundation (Germany) and the Deutsche Forschungsgemeinschaft (German Research Foundation, Co 749/1-1). ENEL Greenpower and the Smithsonian Tropical Research Institute provided logistic support and housing at Fortuna and the Autoridad Nacional del Ambiente (ANAM) provided research permits to undertake the study.

LITERATURE CITED

Adamek M, Corre MD, Hölscher D. **2009**. Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, panama. *Journal of Tropical Ecology* **25**, 637-647.

Andersen KM, Corre MD, Turner BL, Dalling JW. **2010**. Plant–soil associations in a lower montane tropical forest: Physiological acclimation and herbivore-mediated responses to nitrogen addition. *Functional Ecology* **24**, 1171-1180.

Aragão L, Malhi Y, Metcalfe D, Silva-Espejo JE, Jiménez E, Navarrete D, Almeida S, Costa A, Salinas N, Phillips OL. **2009**. Above-and below-ground net primary

productivity across ten amazonian forests on contrasting soils. *Biogeosciences* **6**, 2759-2778.

Burghouts T, Van Straalen N, Bruijnzeel L. **1998**. Spatial heterogeneity of element and litter turnover in a bornean rain forest. *Journal of Tropical Ecology* **14**, 477-506.

Bates, D., Maechler, M., Bolker, B., and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://CRAN.R-project.org/package=lme4

Chalot M, Brun A. **1998**. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiology Reviews* **22**, 21-44.

Chambers JQ, dos Santos J, Ribeiro RJ, Higuchi N. **2001**. Tree damage, allometric relationships, and above-ground net primary production in central amazon forest. *Forest Ecology and Management* **152**, 73-84.

Chave J, Navarrete D, Almeida S, Alvarez E, Aragão LE, Bonal D, Châtelet P, Silva-Espejo J, Goret J-Y, Hildebrand Pv. **2010**. Regional and seasonal patterns of litterfall in tropical south america. *Biogeosciences* **7**, 43-55.

Clark DA, Brown S, Kicklighter DW, Chambers JQ, Thomlinson JR, Ni J, Holland EA. **2001**. Net primary production in tropical forests: An evaluation and synthesis of existing field data. *Ecological Applications* **11**, 371-384.

Cordell S, Goldstein G, Meinzer F, Vitousek P. **2001**. Regulation of leaf life-span and nutrient-use efficiency of metrosideros polymorpha trees at two extremes of a long chronosequence in hawaii. *Oecologia* **127**, 198-206.

Dalling JW, Heineman K, González G, Ostertag R. Geographic, environmental and biotic sources of variation in the nutrient relations of tropical montane forests.

Dantas M, Phillipson J. **1989**. Litterfall and litter nutrient content in primary and secondary amazonian 'terra firme'rain forest. *Journal of Tropical Ecology* **5**, 27-36.

Dezzeo N, Chacón N. **2006**. Litterfall and nutrient input in undisturbed and adjacent fire disturbed forests of the gran sabana, southern venezuela. *INTERCIENCIA-CARACAS-* **31**, 894.

Escudero A, Del Arco J, Sanz I, Ayala J. **1992**. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. *Oecologia* **90**, 80-87.

Girardin CAJ, Malhi Y, Aragao L, Mamani M, Huaraca Huasco W, Durand L, Feeley KJ, Rapp J, Silva-Espejo JE, Silman M et al. 2010. Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the peruvian andes. *Global Change Biology* **16**, 3176-3192.

Green J, Newbery D. **2002**. Reproductive investment and seedling survival of the mast-fruiting rain forest tree, microberlinia bisulcata a. Chev. *Plant Ecology* **162**, 169-187.

Grubb PJ, Metcalfe DJ, Grubb EA, Jones GD. **1998**. Nitrogen-richness and protection of seeds in australian tropical rainforest: A test of plant defence theory. *Oikos*, 467-482.

Henkel TW, Mayor JR, Woolley LP. **2005**. Mast fruiting and seedling survival of the ectomycorrhizal, monodominant dicymbe corymbosa (caesalpiniaceae) in guyana. *New Phytologist* **167**, 543-556.

Hodge A, Campbell CD, Fitter AH. **2001**. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**, 297-299.

Hölscher D, Mackensen J, Roberts J. **2004**. 24 forest recovery in the humid tropics: Changes in vegetation structure, nutrient pools and the hydrological cycle. *This page intentionally left blank*, 598.

Homeier J, Hertel D, Camenzind T, Cumbicus NL, Maraun M, Martinson GO, Poma LN, Rillig MC, Sandmann D, Scheu S. **2012**. Tropical andean forests are highly susceptible to nutrient inputs—rapid effects of experimental n and p addition to an ecuadorian montane forest. *PLoS One* **7**, e47128.

Ichie T, Nakagawa M. **2013**. Dynamics of mineral nutrient storage for mast reproduction in the tropical emergent tree dryobalanops aromatica. *Ecological research* **28**, 151-158.

Kitayama K, Aiba SI. **2002**. Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus pools on mount kinabalu, borneo. *Journal of Ecology* **90**, 37-51.

Koehler B, Corre MD, Veldkamp E, Wullaert H, Wright SJ. **2009**. Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biology* **15**, 2049-2066.

Koerselman W, Meuleman AFM. **1996**. The vegetation n:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* **33**, 1441-1450.

Kuznetsova, A., Brockhoff, P. B., and R. Christensen. 2014. lmerTest: Tests for random and fixed effects for linear mixed effectmodels (lmer objects of lme4 package). R package version 2.0-6. http://CRAN.R-project.org/package=lmerTest

Kuo J, Hocking P, Pate J. **1982**. Nutrient reserves in seeds of selected proteaceous species from south-western australia. *Australian journal of Botany* **30**, 231-249.

Leigh EG. 1999. *Tropical forest ecology: A view from barro colorado island: A view from barro colorado island*: Oxford University Press, USA.

Moser G, Leuschner C, Hertel D, Graefe S, Soethe N, Iost S. **2011**. Elevation effects on the carbon budget of tropical mountain forests (s ecuador): The role of the belowground compartment. *Global Change Biology* **17**, 2211-2226.

Phillips RP, Brzostek E, Midgley MG. **2013**. The mycorrhizal-associated nutrient economy: A new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist* **199**, 41-51.

Proctor, J. 1983. Tropical forest litterfall: problems of litter comparison. In Sutton, S. L., Whitmore, T. C., and A. C. Chadwick (Eds). Tropical rain forest: ecology and management, pp 2676-273. Blackwell, Oxford, UK.

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raich JW, Russell AE, Vitousek PM. **1997**. Primary productivity and ecosystem development along an elevational gradient on mauna loa, hawai'i. *Ecology* **78**, 707-721.

Read D, Perez-Moreno J. **2003**. Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytologist* **157**, 475-492.

Röderstein M, Hertel D, Leuschner C. **2005**. Above-and below-ground litter production in three tropical montane forests in southern ecuador. *Journal of Tropical Ecology* **21**, 483-492.

Satterthwaite FE. **1946**. An approximate distribution of estimates of variance components. *Biometrics bulletin* **2**, 110-114.

Sayer EJ, Wright SJ, Tanner EV, Yavitt JB, Harms KE, Powers JS, Kaspari M, Garcia MN, Turner BL. **2012**. Variable responses of lowland tropical forest nutrient status to fertilization and litter manipulation. *Ecosystems* **15**, 387-400.

Silver WL. **1994**. Is nutrient availability related to plant nutrient use in humid tropical forests? *Oecologia* **98**, 336-343.

Tanner E, Kapos V, Franco W. **1992**. Nitorgen and phosphorus fertilization effects on venezuelan montane forest trunk growth and litterfall. *Ecology* **73**, 78-86.

van Schaik C, Mirmanto E. **1985**. Spatial variation in the structure and litterfall of a sumatran rain forest. *Biotropica*, 196-205.

Veneklaas EJ. **1991**. Litterfall and nutrient fluxes in two montane tropical rain forests, colombia. *Journal of Tropical Ecology* **7**, 319-336.

Villela DM, Proctor J. **1999**. Litterfall mass, chemistry, and nutrient retranslocation in a monodominant forest on maraca island, roraima, brazil1. *Biotropica* **31**, 198-211.

Vitousek PM. **1984**. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **65**, 285-298.

Wolf JH. **1993**. Diversity patterns and biomass of epiphytic bryophytes and lichens along an altitudinal gradient in the northern andes. *Annals of the Missouri Botanical Garden*, 928-960.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE et al. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616-1625.

Table 2.1 Compositional, structural and environmental characteristics of the five one-hectare permanent forest inventory sites. Sites are ordered by increasing soil fertility. Rainfall data are means from 2007-2013. Dry-season rainfall covers 1 January - 30 April. Soil variables were measured at 13 locations per plot in the top 10 cm of soil (Andersen et al. 2009, 2012) and are presented in volumetric units to account for variation in bulk density among sites. Litter data ($\pm$ 1 SD).

Site	Chorro	Honda[1]	Samudio	Palo Seco	Hornito
Substrate	Rhyolitic tuff	Rhyolitic tuff	Andesite	Andesite	Dacite
Dominant species	*Colpothrinax aphenopetala*	*Oreomunnea mexicana*	Mixed forest	Mixed forest	Mixed forest
Basal Area (m^2)	34.2	42.2	39.7	32.5	52.9
% Ectomycorrizal basal area	7%	24%	1%	0%	3%
% Palm basal area	42%	0.5%	0.5%	2%	0%
Elevation (m)	1100	1074	1232	878	1330
Annual temperature (^oC)	20.5	20.2	19.7	21.8	19.2
Annual rainfall (mm)	5434	7246	5105	6032	5477
Proportion of rainfall in dry season (mm)	26%	22%	17%	29%	15%
Soil pH	3.91	4.63	5.06	5.08	5.76
Soil inorganic N (μg N/cm^3)	0.63	3.40	1.42	2.90	4.52
NH_4:NO_3	5.47	4.65	7.09	10.30	10.08
Nitrification rate	0	-0.03	0.10	0.29	0.12
Mehlich extractable soil P	2.74	1.70	3.67	3.91	10.92
Litter N (% N)	1.18 (0.15)	1.39 (0.14)	1.38 (0.22)	1.81 (0.22)	1.66 (0.14)
Litter C:N	38.0 (4.9)	33.0 (3.6)	32.4 (4.6)	24.5 (3.3)	27.1 (2.8)

[1]Honda A in Andersen *et al.* (2010)

Table 2.2 ANOVA table from repeated measures mixed-effects models testing if components of monthly litterfall accumulation differed between the dry and wet season in the 5 1-ha plots examined at Fortuna Forest Reserve. Denominator degrees of freedom were approximated using the Satterthwaite approximation.

	Site Effect		Season Effect		Site x Season	
Litterfall Component	$F_{4,60}$	P	$F_{1,190}$	P	$F_{4,190}$	P
Total	2.89	**< 0.001**	9.77	**< 0.001**	3.06	**< 0.001**
Leaves	16.11	**< 0.001**	157.44	**< 0.001**	14.35	**< 0.001**
Reproductive Tissue	3.34	**0.015**	5.31	**0.022**	1.63	0.169
Support Tissue	2.57	**0.047**	55.95	**< 0.001**	3.42	**0.001**

Table 2.3 ANOVA table from the two-way anova testing the effects of treatment (control vs. nitrogen addition) and year (2009 vs. 2010) on annual litterfall accumulation in the NITROF plots at Fortuna Forest Reserve.

	Treatment Effect		Year Effect	
Litterfall Component	$F_{1,13}$	P	$F_{1,13}$	P
Total	5.95	**0.029**	0.42	0.529
Leaves	3.08	0.102	0.36	0.314
Reproductive Tissue	0.07	0.798	0.58	0.462
Reproductive:Leaf Ratio	0.41	0.532	0.59	0.456

Figure 2.1 Study sites in the Fortuna Forest Reserve and adjacent Palo Seco reserve in western Panama. The five sites were located on three geological types with contrasting soil fertility. Palo Seco and Samudio are intermediate fertility sites on Andesite; Honda and Chorro are low fertility sites on Rhyolite; Hornito is a high fertility site on Dacite. A gradient in increasing seasonality of rainfall goes approximately from N-S from Palo Seco on the Caribbean slope of the continental divide, to Hornito.

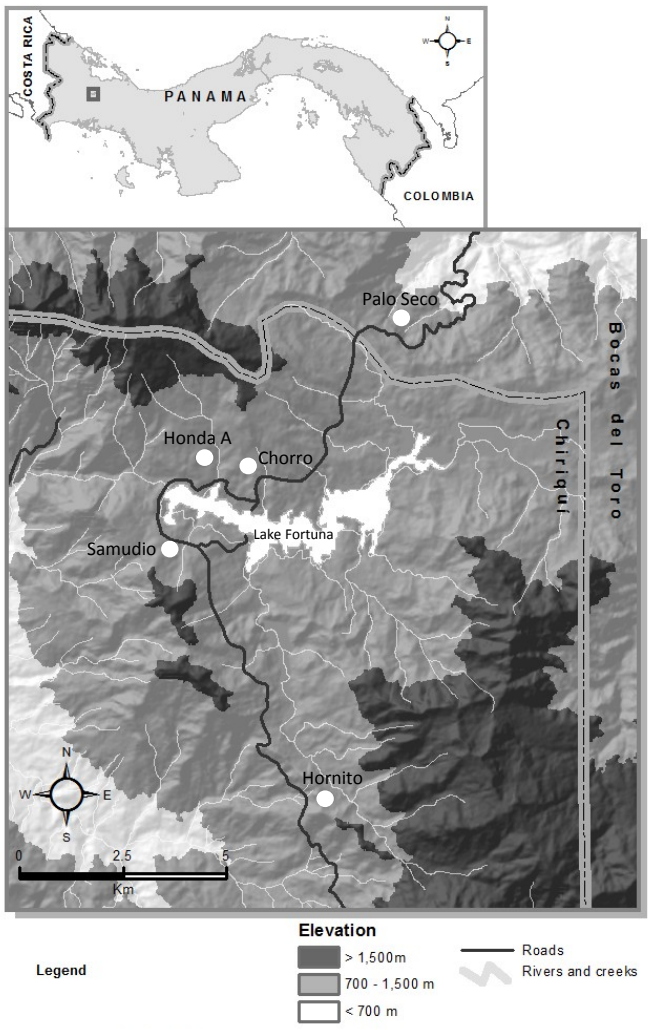


Figure 2.2 (A) Mean annual litterfall (Mg/ha/yr) measured over the calendar years 2009 and 2010 at each of the site ordered by increasing soil fertility. Stacked bars represent the contributions of each litter fraction (support = wood, bark, and epiphytes); error bars are standard errors calculated on total litterfall. (B) Mean annual litterfall adjusted for differences in basal area among plots ($Mg/m^2_{basal\ area}/yr$).

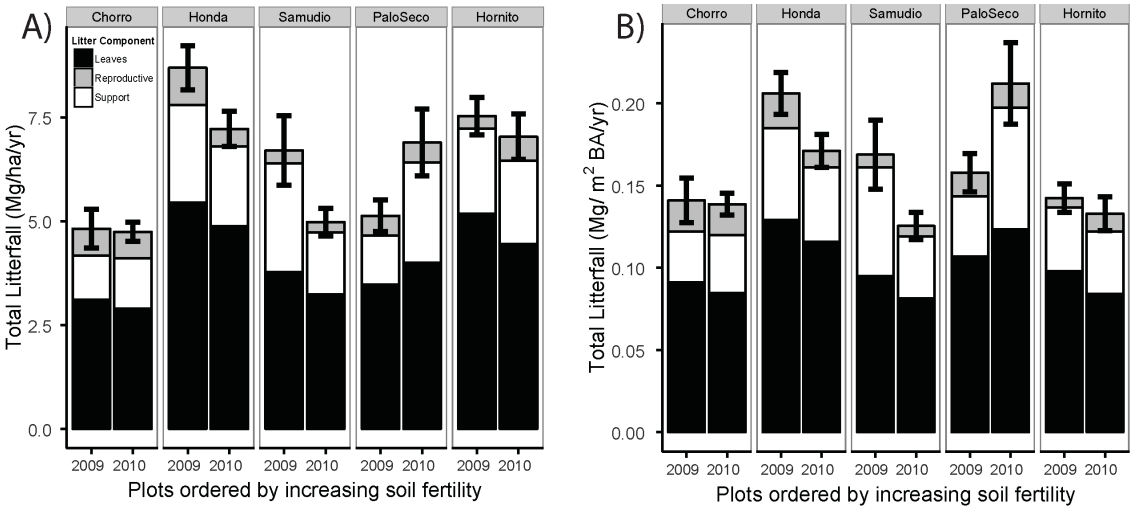


Figure 2.3 (A) Mean annual nitrogen (N) deposited by litterfall (kg/ha/yr) and (B) ratio of reproductive to leaf litter biomass among sites in 2009 and 2010.

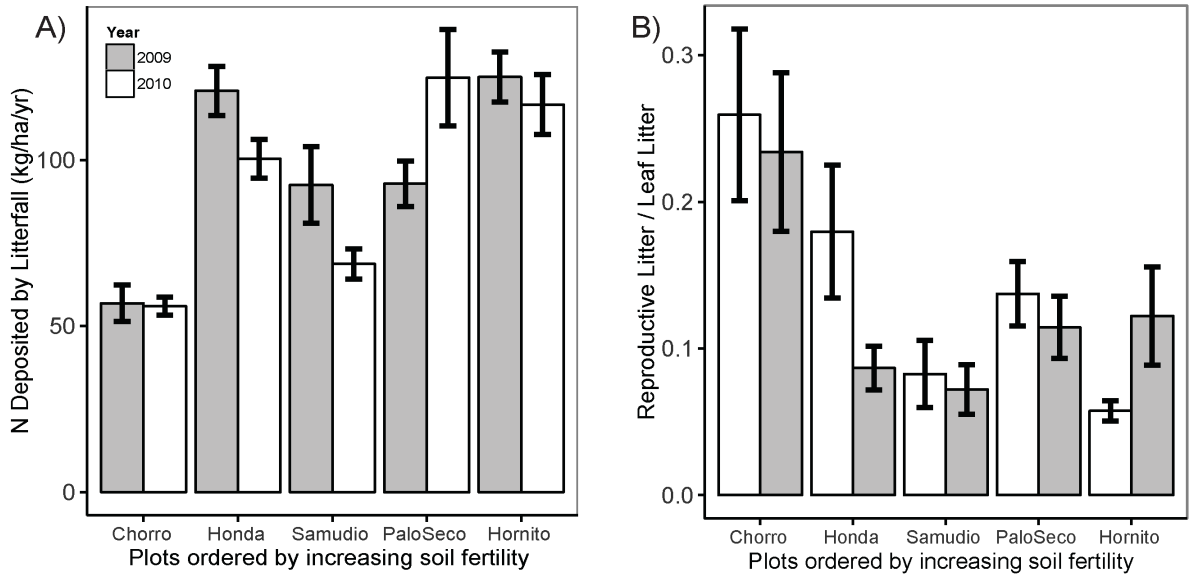


Figure 2.4 Across-site comparison of total litterfall biomass over the two-year collection period. The litter collected following the severe storm in February 2009 accounted for 25 percent of the litter biomass measured that year.

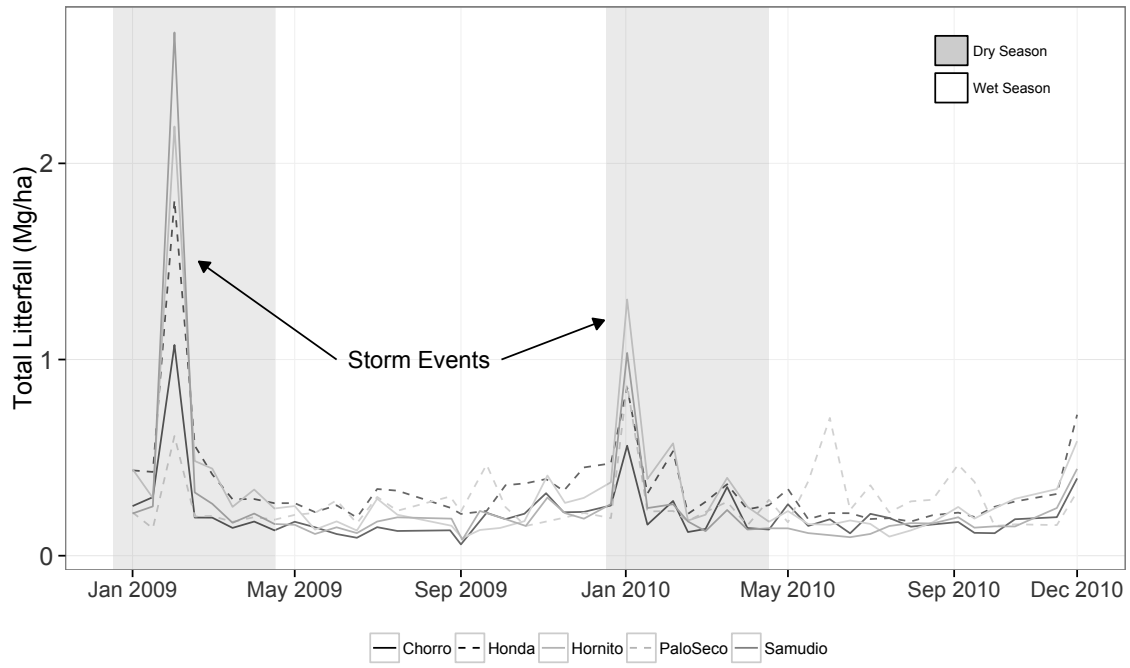


Figure 2.5 Mean dry and wet season total (A), leaf (B), reproductive (C), and support (D) monthly litterfall accumulation ± 1 SE in each site. "*" indicates a significant difference in litterfall accumulation between seasons ($P < 0.05$).

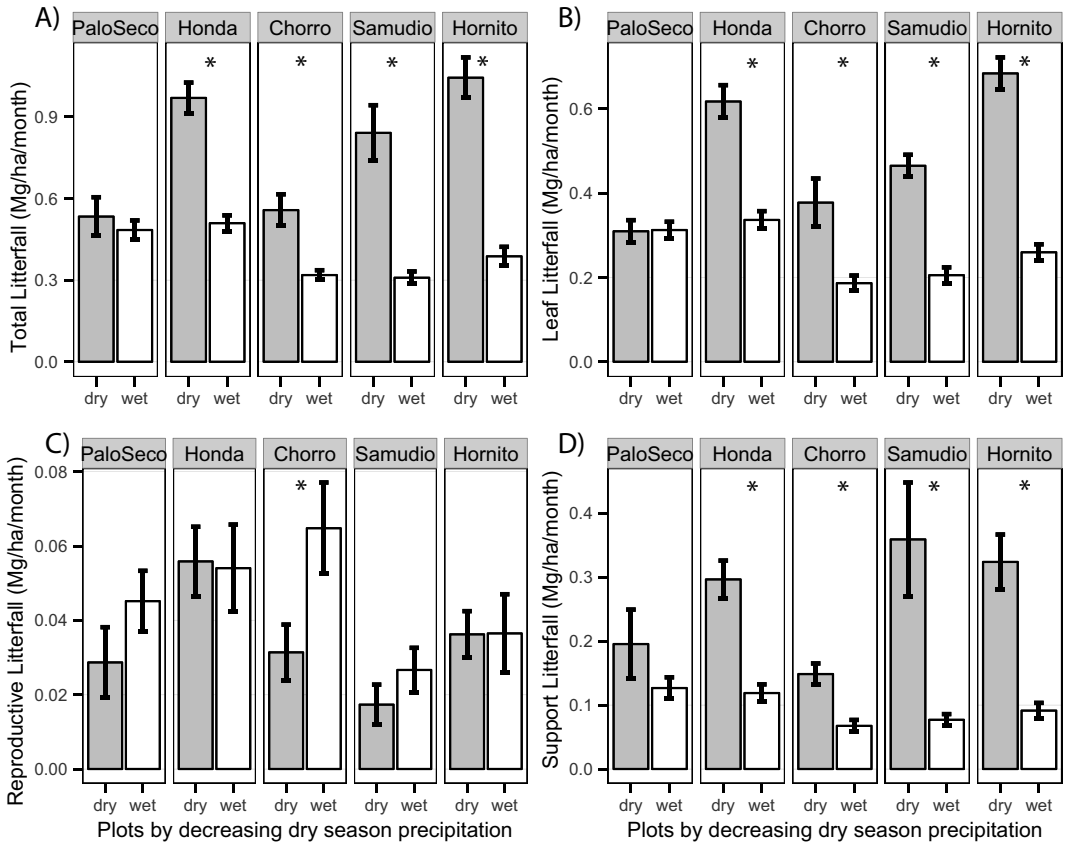
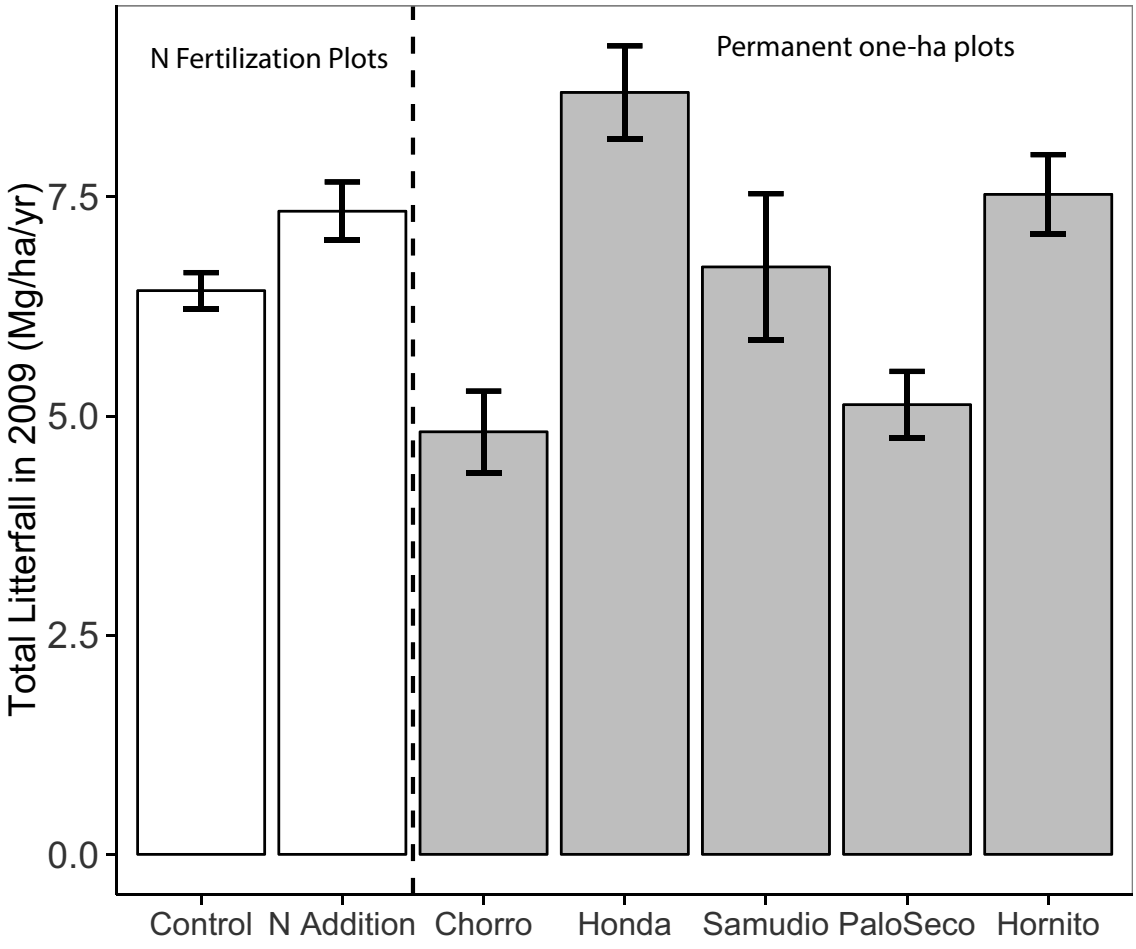


Figure 2.6 Mean total litterfall (Mg/ha/yr) in control and nitrogen-addition plots in the on-going nitrogen addition experiment in fourth year of the experiment (2009) compared to the variation in total litterfall accumulation in the same year among the five permanent 1-ha plots spanning the Fortuna soil fertility gradient.



CHAPTER 3[2]

VARIATION WOOD NUTRIENTS ALONG A TROPICAL SOIL FERTILITY GRADIENT

ABSTRACT

Wood contains the majority of the nutrients in tropical trees, yet controls over wood nutrient concentrations and their function are poorly understood. We measured wood nutrient concentrations in 106 tree species in 10 forest plots spanning a regional fertility gradient in Panama. For a subset of species, we quantified foliar nutrients and wood density to test whether: 1) wood nutrients scale with foliar nutrients at the species level, or 2) wood nutrient storage increases with wood density as predicted by the wood economics spectrum. Wood nutrient concentrations varied enormously among species from 4-fold in nitrogen (N) to > 30-fold in calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P). Community-weighted mean wood nutrient concentrations correlated positively with soil Ca, K, Mg, and P concentrations. Wood nutrients scaled positively with leaf nutrients, supporting the hypothesis that nutrient allocation is conserved across plant organs. Wood P was most sensitive to variation in soil nutrient availability, and significant radial declines in wood P indicated that tropical trees retranslocate P as sapwood transitions to heartwood. Wood P decreased with increasing wood density, suggesting that low wood P and dense wood are traits associated with tree species persistence on low fertility soils. Substantial variation among species and communities in wood

[2] **Heineman, K. D.**, Turner, B. L., and J W. Dalling. 2016. Variation in wood nutrients along a tropical soil fertility gradient. New Phytologist. DOI: 10.1111/nph.13904.

nutrient concentrations suggests that allocation of nutrients to wood, especially P, influences species distributions and nutrient dynamics in tropical forests.

INTRODUCTION

Tropical rainforests commonly occur on strongly weathered soils depleted in rock-derived nutrients such as calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P). As a consequence, productivity in tropical forests is assumed to be limited by the availability of one or more soil nutrients (Vitousek, 1984; Tanner *et al.*, 1998; Wright *et al.*, 2011). In addition to effects on productivity, spatial heterogeneity in soil nutrient availability can drive compositional variation in tropical tree communities at local scales and regional scales (John *et al.*, 2007; Baldeck *et al.*, 2013; Condit *et al.*, 2013). Differences among species in nutrient acquisition and allocation potentially underlie soil-based habitat partitioning in tropical forests (Chapin, 1980; Fine *et al.*, 2004; Palmiotto *et al.*, 2004; Baltzer *et al.*, 2005; Kelly M. Andersen *et al.*, 2012), and exacerbate plant-soil feedbacks that influence nutrient availability via nutrient deposition and mineralization from plant material (Wardle *et al.*, 2004). Consequently, quantifying variation in tree species allocation of limiting nutrients over soil fertility gradients is critical to understanding community assembly and biogeochemistry in tropical forests.

Tree nutrient-use strategies are most commonly evaluated through the nutrient stoichiometry of fresh leaves. Soil-based habitat distributions have been linked to foliar nutrient concentrations, because tropical plant taxa associated with high fertility soils contain greater concentrations of N and P in fresh leaves than species associated with low fertility soils (Edmund V. J. Tanner, 1977; Andersen *et al.,* 2012; Katabuchi *et al.*, 2012; Dalling *et al.,* 2015). Despite shifts in community mean foliar chemistry along soil fertility gradients (Vitousek *et al.*, 1988; Han *et al.*, 2005; Ordoñez *et al.*, 2009; Fyllas *et al.*, 2009; Hayes *et al.*, 2014), foliar nutrient

concentrations are poorly constrained by soil nutrient availability in tropical forests, as variation in foliar nutrients among co-occurring species growing on the same soil habitat is nearly as great as regional variation in species foliar nutrients across soil fertility gradients (Townsend *et al.*, 2007; Fyllas *et al.*, 2009). Given the taxonomic control over foliar nutrient concentrations and the importance of tissue N and P concentrations in predicting rates of leaf (Santiago, 2007; Bakker *et al.*, 2011) and wood (Weedon *et al.*, 2009; Zanne *et al.*, 2015) decomposition, a trait-based approach leveraging taxon-specific chemical attributes has been advocated for modeling global carbon turnover from leaf (Cornwell *et al.*, 2008) and woody (Cornwell *et al.*, 2009) biomass. While access to species level information is rapidly expanding from to the emergence of global trait databases (Kattge *et al.*, 2011), chemical attributes of wood are poorly represented in the literature (Chave *et al.*, 2009), despite wood containing roughly half of Ca, K, Mg, N, and P in live vegetation in tropical forests (Tanner, 1985; Wang *et al.*, 1991; Stanley & Montagnini, 1999; Bond, 2010). Trait inventories evaluating tropical tree species wood nutrient concentrations, in concert with more widely collected foliar and woody attributes, are necessary to more fully understand the variability among species in whole tree nutrient use and the implications of this variability for ecosystem processes.

The extent to which foliar traits can be used to predict woody traits may depend on the range of soil habitats and taxonomic groups represented in the analysis. For instance, a global meta-analysis of 145 woody species found that stem and leaf nutrient concentrations were significantly, albeit weakly, correlated for both N ($r^2 = 0.20$) and P ($r^2 = 0.22$; Kerkhoff *et al.*, 2006). However, this pattern may be driven in part by latitudinal gradients in soil N and P availability, which are reflected in foliar nutrient concentrations (Reich & Oleksyn, 2004), rather than constraint across organs in nutrient allocation at the species level. While decomposition

rates of species wood, leaf, and root tissues are correlated both within sites and across global scales (Freschet *et al.*, 2013), species wood and leaf decomposition rates are decoupled when angiosperm and gymnosperm taxa are analyzed separately (Pietsch *et al.*, 2014), suggesting that the allocation of nutrients and secondary metabolites might not be constrained across organs in co-occurring angiosperms. Furthermore, a multivariate analysis of wood and leaf characteristics in neotropical tree species found that physical wood and leaf traits load along orthogonal axes of variation (Baraloto *et al.*, 2010), indicating that the selection pressures that shape species tradeoffs among foliar traits might differ from those controlling the evolution of woody traits. Consequently, while we expect that species wood and leaf nutrient concentrations should be correlated at global scales, this relationship is not well understood in highly diverse tropical tree communities.

Wood and leaves differ in functional attributes that might disrupt a tight correlation between nutrient concentrations of these organs. Whereas woody biomass could provide a well-defended storage organ for nutrients, water, and carbohydrates (Chapin *et al.*, 1990), leaves are a poorer storage organ due to their short lifespan and vulnerability to herbivores. Susceptibility to herbivory increases with foliar N concentration (Mooney & Gulmon, 1982; Andersen *et al.*, 2010), thereby creating a possible constraint on maximum investment of N in leaf metabolism, with consequences for other nutrients that are linked through stoichiometry. Compared to young, fully expanded leaves, stem nutrient concentrations display a greater relative increase in N and P concentrations in response to experimental N and P addition in seedlings in Panama (Schreeg *et al.*, 2014) and trees in China (Mo *et al.*, 2015), which may reflect a greater capacity for nutrient storage in wood compared with leaves. A global meta-analysis of 71 angiosperm species found not only that sapwood Ca, K, Mg, N, and P concentrations all vary by an order of magnitude, but

also, species vary considerably in the ability to resorb nutrients as sapwood transitions to heartwood (Meerts, 2002). Given the potential variation in nutrient storage and remobilization in woody tissues among species, wood nutrients may constitute an important dimension of functional variation in tropical tree communities.

The scarcity of interspecific wood nutrient data limits our understanding of co-variation between wood nutrients and other plant functional traits in tropical forests. This paucity of information was acknowledged in the development of the wood economics spectrum (WES; Chave *et al.*, 2009), a comprehensive meta-analysis emphasizing the inverse relationship between tree species wood density and mortality rates. The WES predicts tradeoffs among wood traits that facilitate fast growth (large conduit diameter) and adaptations that promote survival (high wood density, high storage capacity). In line with these predictions, stem nonstructural carbohydrate storage has been linked to increased wood density and decreased mortality rates in tropical forest saplings (Poorter & Kitajima, 2007). To date, studies evaluating the relationship between wood chemistry and the growth–survival axis have been limited to wood N and provide mixed evidence: wood N correlated positively with wood density and negatively with relative growth rate for 54 tree species in Panama (Martin *et al.*, 2014), while no relationship was found between the density and N content of wood in 23 tree species in Uganda (Becker *et al.*, 2012). Given that species leaf N concentration correlates negatively with wood density (Kraft *et al.*, 2008) and positively with species diameter growth rates in tropical forests (Poorter & Bongers, 2006), a positive relationship between wood nutrients and wood density would signal substantial functional decoupling among plant organs. To better understand how components of species nutrient use strategies relate to life history tradeoffs, wood nutrients must be evaluated with

respect to foliar nutrients and wood density in co-occurring species in high diversity tropical forests.

Here, we measured the concentrations of Ca, K, Mg, N, and P in the wood of 106 Panamanian tree species growing in 10 montane and lowland forests spanning a range of soil 'available' nutrient concentrations comparable to the range observed throughout the tropics (Gartlan *et al.*, 1986; Baillie *et al.*, 1987; Phillips *et al.*, 2003, Quesada *et al.*, 2009). By exploiting this extreme soil gradient, we present the most robust examination to date of the natural variation in wood nutrient concentrations among species and sites in tropical forests. We hypothesized that if taxonomic variation in nutrient allocation influences the distribution of tree species across soil fertility gradients, then (i) community mean wood nutrient concentrations should correlate with soil nutrient availability, and (ii) there should be substantial interspecific variation in wood nutrient concentrations within a site. We also evaluated whether wood nutrient concentrations in 58 montane forest species correlate with other species-specific functional traits, including foliar nutrients and wood density. If nutrient allocation to biomass is constrained across plant organs at the species level, then species wood nutrient concentrations should increase with species leaf nutrient concentrations, which are often negatively correlated with wood density. Alternatively, we hypothesized that if wood nutrient concentrations are proportional to the investment of nutrients into storage reserves, then tree species wood nutrient concentrations should correlate positively with wood density, which is associated with persistence and survival strategies in tropical tree species.

MATERIALS AND METHODS

Study site

We sampled foliar and woody tissue from six montane forest sites located within the Fortuna Forest Reserve (19,500 ha) and the adjacent Palo Seco Forest Protectorate (125,000 ha), henceforth Fortuna, in western Panama (Figure 3.1). This region encompasses old growth, lower montane forest, ranging between 700 and 1500 m asl, with mean annual temperatures varying between 19 and 23°C (Cavelier *et al.*, 1997). There is strong interannual and spatial variability in precipitation among study sites, with annual rainfall ranging from 4000 to 9000 mm per year. A distinct dry season occurs from January to April, but evapotranspiration does not exceed rainfall during this period (Cavelier *et al.*, 1997), with monthly rainfall accumulation exceeding 100 mm per month on average during the dry season in all but one site (Table 3.1).

Twelve permanent 1-ha forest plots were established at Fortuna in 2003 in which all trees > 5 cm diameter at breast height (DBH) are mapped, measured, and identified to species. Plant tissue was sampled in six plots, chosen to maximize variation in soil nutrient availability across three geological substrates: rhyolitic tuff, andesite, and porphyritic dacite (Andersen *et al.*, 2010). Soil pH ranges between 3.6 and 5.6 among sites, which coincides with substantial variability in Ca, K, Mg, N, and P (Table 3.1). There is considerable floristic turnover among soil habitats, with only 22% of species shared between soils developed on dacite and rhyolite located < 15 km apart.

We sampled wood from four additional lowland sites in the Panama Canal watershed, part of a network of 1-ha plots established in the region by the Center for Tropical Forest Science (Figure 3.1; Pyke *et al.*, 2001; Turner & Engelbrecht, 2011; Condit *et al.*, 2013). Two of the focal plots were located on peninsulas adjoining the Panama Canal (P13 and P25), and two plots

in Soberanía National Park were located along Pipeline Road (P06) and Camino de Cruces (P24; plot codes from Pyke *et al.*, 2001). This region consists of semi-deciduous, seasonally moist forest, receiving approximately 2500 mm of annual rainfall and with a mean annual temperature of 27°C (Pyke *et al.*, 2001). In the dry season, periods during which evapotranspiration exceeds rainfall are frequent and influence the recruitment of species in this area (Engelbrecht *et al.*, 2006). Focal plots are classified as mature secondary forest aged between 60-100 years (Pyke *et al.*, 2001). Canal watershed plots were selected to maximize contrast along the soil fertility gradient (Table 3.1). The underlying geological substrates of the plots in order of increasing P availability are: rhyolitic tuff (P25), Gatuncillo Formation (marine sediment, P06), Caimito Formation (marine sediment, P13), and agglomerate (P24) (Turner & Engelbrecht, 2011).

Plant tissue sampling and analysis

Wood core samples were extracted from 301 individual trees from 76 species at Fortuna and 104 trees from 30 species the Canal Watershed. In all plots, we sampled 7-22 woody species with the greatest basal area in each plot. We cored three trees > 10 cm DBH per species using a 4.3 mm Haglöf increment borer. Cores were taken at breast height (1.3 m) to a depth of half the DBH of the tree. Because coring canopy palms at Fortuna with hard exteriors and soft interiors damaged the borers, palms were not sampled in the Panama Canal Watershed. Consequently, compared to other sites, the sampled species represented a smaller proportion of plot basal area in P06 and P24 (Table 3.1) where the palm genera *Astrocaryum*, *Oenocarpus*, and *Attalea* make up > 20% of the total basal area. Trees at Fortuna were cored outside permanent forest plots, but within 100 m of the plot boundary in February 2011. Lowland wood samples were collected from trees located within 1-ha plots in July 2013. At Fortuna, foliar tissue was collected for 58 of the species sampled for wood in July 2010. Three fully expanded shade leaves were collected

with a pruning pole from three individuals per species. While functional trait protocols recommend the collection of sun leaves for foliar nutrient analysis (Cornelissen *et al.*, 2003), we sampled shade leaves to maximize the number of species included in our study, because 43% of tree species with individuals > 10 cm DBH in the Fortuna plots do not reach the canopy (DBH > 30 cm). Analysis of foliar plasticity to light in 38 tropical tree species found that rank order in species mean N and P concentrations is preserved across sun and shade leaves (Rozendaal *et al.*, 2006), indicating shade leaves should be provide insight into how wood and leaf nutrients co-vary across species. Wood and leaf samples were stored on ice until processing. Each wood core was divided into segments no greater than 5 cm and volume determined by Archimedes' principle. Segments were then dried to constant mass at 60°C and wood density was calculated as segment dry mass / fresh volume.

All leaf material collected for each tree, including petioles and rachii, were ground together in a KLECO Tissue Pulverizer (Kinetic Laboratory Equipment, Visalia, CA). A mini-Wiley Mill (Thomas Scientific, Swedesboro, NJ) was used to grind wood core samples in 5 cm segments to account for possible radial differences in wood attributes (Lachenbruch *et al.*, 2011). We used 5 cm as a critical threshold of change in wood chemistry based on the steep observed decline in nonstructural carbohydrate concentrations between 4.5 and 6 cm in wood cores taken from lowland Panamanian tree species (Würth *et al.*, 2005). We were unable to categorize segments visually as heartwood or sapwood because the heartwood/sapwood transition is often gradual in tropical trees (Jordan & Kline, 1977). Nitrogen concentrations in leaf and wood tissues were tested on a Costech Elemental Analyzer (Valencia, CA) for samples analyzed in Illinois and a Thermo Flash 1112 Elemental Analyzer (Waltham, MA) for samples analyzed in Panama. A subset of samples tested on both Elemental Analyzers also closely and linearly

correlated (r^2 = 0.92, n=10), although a correction factor of -0.18 was applied to all samples tested in Panama to ensure consistency in the two datasets. To prepare wood and leaf material for Ca K, Mg, and P analysis, samples were dry ashed at 550°C for 1 hour and the ash dissolved in 1 M HNO_3 (Karla, 1998). Base cations for all samples and P in tissues collected at Fortuna were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) on an Optima 2000 DV (PerkinElmer, Waltham, MA). A subset of Fortuna wood samples with P concentrations below the ICP-OES detection limit and all lowland wood samples were analyzed for P via automated molybdate colorimetry using the Lachat Quickchem 8500 (Hach Ltd., Loveland, CO). For samples above the detection limit of the ICP analyzed on both instruments, P measurements by spectrometry and colorimetry were closely correlated (r^2 = 0.95, n=10) with an intercept that did not differ significantly from zero. We included certified reference samples (NIST 1515, apple leaves) and internal laboratory control standards in all analyses.

Soil sampling and analysis

Soil cores were taken to a depth of 10 cm from 13 locations in each 1-ha plot during the wet season at both lowland and montane sites. Bulk density was determined by drying a known volume of soil at 105°C. Soil pH was determined in a 1:2 soil to deionized water ratio using a glass electrode. Total soil inorganic N was calculated as the sum of soil nitrate and ammonium measured in 0.5 M K_2SO_4 extracts and determined by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Readily exchangeable P, which approximates plant available P, hereafter "resin P", was determined by extraction with anion-exchange membranes (Turner & Romero, 2009). Base cations were extracted in Mehlich-3 solution (Mehlich, 1984) with detection by ICP–OES on an Optima 7300 DV spectrometer (Perkin-Elmer, Shelton, CT).

Statistical methods

Response of site-mean wood chemistry to soil nutrient availability

For consistency, we limited our analyses of plot and interspecific variation in wood nutrient concentrations and densities to data from the outer 5 cm of wood because the majority of trees cored were not big enough to yield multiple 5 cm segments. The community weighted mean (CWM) nutrient concentration for each 1-ha plot was calculated as the average of species mean wood nutrient concentrations (in the outer 5 cm annulus) weighted by basal area of each species sampled in the 1-ha plot (trees > 10 cm DBH). Ordinary least squares (OLS) regression was used to fit the relationship between CWM wood and plot mean soil nutrient concentrations, and between species mean wood nutrient concentrations and respective plot level soil nutrient concentrations to determine how much variation in species wood nutrient concentrations is explained by soil nutrients alone. Wood nutrient concentrations and soil variables were log transformed prior to regression analyses to meet the assumption of normality of errors.

Wood vs. Leaf Scaling Relationships

We modeled the relationship between species mean wood and leaf nutrients among organs for 58 woody species sampled for both leaf and wood nutrients at Fortuna. If a species was sampled in more than one plot, we used the mean species value across all plots so that there were no duplicate species in the analysis. We modeled wood-leaf scaling as power functions ($Y \sim aX^b$), which was transformed to be evaluated as a linear relationship ($\log(Y) \sim b*\log(X) + \log(a)$). If exponent or slope of this relationship (b) differs from one, then scaling is not nonlinear, indicating that the nutrient concentration of one tissue is more constrained in one tissue versus the other. We fit the log-log relationship between species mean leaf and wood values for each element using type II Major Axis (MA) regression in the *lmodel2* package in R (Legendre,

2011). MA regression is recommended over OLS regression in this case because there is similar measurement error in both variables. We used 95% confidence intervals to determine if b differed significantly from 1. Because we modeled mean wood nutrient concentrations as a function of leaf nutrient concentrations, $b > 1$ indicates that leaf nutrients are more constrained among species than wood nutrients. We determined r^2 values from OLS regression of each relationship to find the proportion of variance in species mean wood nutrient concentrations explained by foliar nutrient concentrations.

Because some species are more closely related in evolutionary history than others, species trait values may be considered non-independent and therefore violate the assumption of linear regression. To determine if species wood and leaf nutrient concentrations co-vary after accounting for evolutionary history, we tested relationships for the phylogenetically independent contrasts (PICs) of log-transformed species mean wood and leaf nutrient concentrations (Felsenstein, 1985). We constructed a phylogenetic tree for the species in our study from the Angiosperm Phylogeny Group super tree (APG III; http://www.mobot.org/MOBOT/research/APweb) using Phylomatic version 3.0 (Webb & Donoghue, 2005). We used the fossil-derived ages of tree taxa listed in Wikstrom (Wikström *et al.*, 2001) to determine the branch lengths of this tree using BLADJ in Phylocom (Webb *et al.*, 2008). Because the APG III tree is resolved to the family level for most lineages, genera were drawn as polytomies nested within families and species were drawn as polytomies within genera. Prior to phylogenetic analyses, polytomies in the tree were broken randomly. PICs for the log of each species trait value were calculated using the *ape* package (Paradis *et al.*, 2004) in R. We used MA regression to fit the scaling relationship between the PICs of wood and leaf nutrient concentrations. PIC models were fit through the origin as suggested in Garland *et al.*, (1992).

Wood Nutrient Concentrations versus Wood Density

For 76 woody species cored at Fortuna, we tested if species mean wood nutrient concentrations co-vary with each other and with woody density using MA regression. We log transformed wood nutrient concentrations prior to regression analysis to meet the assumption of normality of errors. We performed the same regression analysis for the PICs of log-transformed species mean wood nutrients and non-log transformed wood density. The significance threshold of regression tests was adjusted using the Bonferroni correction to account for multiple comparisons. If a species was cored in more than one plot, we used the average species mean across all plots so that there were no duplicate species in the analysis.

Radial Variation in Wood Nutrient Concentrations

For the 110 trees, we fit the log-log relationship between the nutrient concentrations of the inner (5-10 cm in depth) vs. outer (0-5 cm in depth) wood annuli for each element using MA regression. We used 95% confidence intervals to determine if the intercept differed from zero and the slope differed from 1. We also calculated the coefficient of determination r^2 from OLS regression to evaluate how well the concentration of inner annuli can be predicted from outer annuli.

We evaluated if species vary in radial patterns of wood nutrient allocation for 18 species for which we had available data on both inner and outer wood segments for ≥ 3 individuals per species. For each tree, calculated the percent radial discrepancy in wood nutrients as: (outer – inner)/ outer * 100. We determined the mean radial discrepancy for each species ± one standard error to determine if the change in wood nutrient concentrations from the outermost segment to adjacent inner segment is < 0, $= 0$, > 0.

RESULTS

Interspecific and inter-site variation in wood nutrients

Of the elements measured in the outer 5 cm of wood across the 106 tree species sampled in this study, mean concentrations of N were greatest (2557 ± 70 μg g^{-1}), followed by Ca (2082 ± 160 μg g^{-1}), K (1622 ± 80 μg g^{-1}), Mg (492 ± 40 μg g^{-1}), and P (111 ± 7 μg g^{-1}; Table 3.2; Appendix A). While wood nutrient concentrations varied considerably among species and sites, the magnitude of this variability was not consistent among elements (Table 3.2). The range in mean wood N among species (five fold) was less than the smallest range of species means measured for rock-derived elements including P (35-fold), K (36-fold), Ca (47-fold), and Mg (51-fold). When species averages were weighted by basal area to calculate CWM nutrient concentrations, there was also greater range of values among sites in wood P and cations compared to wood N (Table 2): N (1.8-fold), Mg (2.5-fold), K (4.5-fold), Ca (7-fold), and P (8.5-fold).

Wood nutrient concentrations vs. soil nutrient availability

In line with our predictions, CWM wood nutrient concentrations (a plot-level estimate of wood nutrient status) for Ca, K, Mg, and P were significantly correlated with the plot mean nutrient concentrations in the topsoil (Fig. 3.2a-c,e). CWM wood P concentration correlated most strongly with its respective soil metric (r^2 = 0.71), followed by Mg (r^2 = 0.64), K (r^2 = 0.58), and Ca (r^2 = 0.49). Although CWM wood N was not significantly correlated with soil inorganic N (r^2 = 0.00, P = 0.519; Fig. 3.2e), soil resin P was a strong predictor of CWM wood N (r^2 = 0.59, P = 0.009), indicating that CWM wood N concentrations varies along the fertility gradient.

When species-level wood nutrient means were modeled as a function of plot-level soil nutrient concentrations (Fig. 2f-j, Table 3.5), the relationship between wood Mg and soil Mg was

no longer significant (r^2 = 0.01, P = 0.141). For all nutrients, the slope of the species means wood vs. soil regression was smaller in magnitude than the slope of the CWM (basal-area weighted) wood vs. soil regression fit (Fig. 2, Table 3.5), suggesting that species with high basal area better reflect local soil nutrient availability than rare or small stature species.

Wood versus leaf nutrient concentrations

For 58 montane tree species for which we analyzed both wood and leaf nutrient concentrations, species mean leaf and wood nutrient concentrations were significantly positively correlated for all elements evaluated, supporting the hypothesis that nutrient allocation is constrained across organs at the species level. Among significant relationships, wood and leaf tissue chemistry was most strongly correlated for P (r^2 = 0.36; Fig. 3.3e) and most weakly correlated for Mg (r^2 = 0.18; Fig. 3.3c). For Ca, K, Mg and P, the slope of the wood vs. leaf relationship (b) was significantly > 1, indicating that wood nutrient concentrations scale nonlinearly with leaf nutrient concentrations (Table 3.3; Fig. 3.3). In contrast, the slope of the leaf vs. wood regression did not differ from 1 for N (Table 3.3; Fig. 3.3d), indicating that N scales isometrically between wood and leaf tissues.

When wood and leaf nutrient concentrations were corrected for evolutionary history using PICs, the scaling relationship remained significant for all nutrients but Mg (Table 3.3). Slopes of PIC models did not differ from observed models for any nutrient (Table 3.3). Scaling exponents of relationship between wood-leaf PICs remained significantly > 1 for K and P, and the b value of the wood–leaf Ca PIC model marginally overlapped with 1 (95% CI = 0.99–2.10).

Wood nutrient concentrations versus wood density

We did not find support for the hypothesis that wood nutrient concentrations increase with wood density. Species mean wood density significantly declined as species mean wood P

increased and was not significantly associated with any other wood nutrient (Table 3.4). Wood Ca, K, Mg, N, and P were all significantly positively correlated with one another at critical α = 0.05 (Table 3.4). After correcting for multiple comparisons, Ca was significantly correlated with only N and P, and Mg was correlated with only P (Table 3.4).

Correlation of the PICs of species woody traits gave qualitatively similar results to analysis of observed values at α = 0.05, except that PIC of wood Mg was negatively correlated with PIC of wood density (Table 3.6). After correcting for multiple comparisons, PIC of wood P remained significantly correlated with all wood traits, however, the relationships of wood Ca, Mg, and N with wood K were no longer significant, and wood Mg was no longer correlated with wood Ca or wood density (Table 3.6).

Radial variation in wood nutrients

In the 110 tree cores examined, the concentration of macronutrients in the outermost 5 cm of wood was strongly positively correlated with nutrient concentrations in the adjacent 5-10 cm segment for all elements (Fig. 4; Table 3.7). Nutrient concentrations in the outer segment explained a greater proportion of the variance than the inner segment for Ca (r^2 = 0.74), Mg (r^2 = 0.77), and N (r^2 = 0.75) compared to K (r^2 = 0.53) and P (r^2 = 0.61). For K, N, and P in MA regression models, intercept of the inner vs. outer relationship was significantly < 0 and the slope was significantly > 1 (Table 3.7), indicating that inner segments have lower concentrations of K, N, and P on average than the outer segments at low element concentrations. However, this discrepancy between segments declines or reverses with increasing element concentration (Fig. 4). For 88 of 110 individuals, the outer core segment had a higher wood P concentration than the inner core segment. The Ca and Mg inner vs. outer regression parameters did not significantly differ from a 1:1 relationship (Table 3.7).

Species varied in the magnitude and the direction of radial differences in nutrient concentrations between outer and inner annuli. Of the elements examined, radial patterns in wood P were most qualitatively consistent across species, as 14 of 18 species had significantly higher P concentrations in the outer versus inner core. Wood P concentrations declined by 35% from the outer to inner segments across all species, although species mean radial P discrepancies ranged widely from 2% to 88% (Fig. 3.5). In contrast, Ca and Mg concentrations were significantly lower in the outer compared to the inner core for the majority of species (Fig. 3.5), and the magnitude of this difference exceeded 50% for four of 18 species. However, qualitatively distinct radial cation differences were present in other species (Fig. 3.5). For wood N and K, there were similar numbers of species with radial differences < 0, $= 0$, or > 0.

DISCUSSION

Variation in wood nutrients among species and sites

Wood nutrient concentrations varied substantially among 106 tree species sampled along a regional soil fertility gradient in Panama. For some nutrients, observed values encompassed the entire range of wood nutrient concentrations reported in tropical tree species to date. Wood N concentrations (Table 2) fell within the range of values reported previously for tropical forest species (400–6900 μg g^{-1}; Mascaro *et al.*, 2012; Becker *et al.*, 2012; Martin *et al.*, 2014). The same was true for the observed range of species mean wood Ca concentrations in this study, which fell within the range of the values reported for sapwood in the Meerts, 2002) meta-analysis of 93 temperate and tropical angiosperm species (60–15000 μg g^{-1}). In contrast, the range in wood P concentrations observed (19–668 μg g^{-1}) here exceeded the range of previously reported for sapwood P concentrations in wild angiosperms (20–615 μg g^{-1}; Meerts, 2002; Mascaro *et al.*, 2012). The range in species wood K and Mg observed here exceeded the

maximum previously reported values for sapwood K (160–4500 μg g^{-1}) and Mg (80–1290 μg g^{-1}) reported in Meerts, 2002). Furthermore, the average of species mean wood Ca, K, and P concentrations in this study all exceeded the "typical range" of wood nutrient concentrations reported in Chave *et al.*, 2009), which is a frequently cited reference for wood traits, indicating that calculations of forest wood nutrient stocks based on this review may be underestimated.

Basal area-weighted (CWM) wood nutrient concentrations also differed widely among plots, and paralleled soil nutrient availability for all elements except N. Because the concentrations of macronutrients strongly covaried in both woody biomass and the soil available pools, it is difficult to determine if the ecosystem sequestration of nutrients in woody biomass is proportional to the soil availability of each nutrient independently, or if the increases in the availability of one limiting element increases the uptake of non-limiting elements to maintain stoichiometric balance (Marschner, 1995). For example, CWM wood N might correlate significantly with soil P, but not soil N, because N investment in wood is constrained by P limitation. In contrast, the Stability of Limiting Nutrients Hypothesis (WX Han *et al.*, 2011) would posit that the low variability in wood N among species and along environmental gradients is evidence that N is more limiting than other elements because increased availability of limiting elements should result in increased growth, not increased tissue nutrient concentrations. Given that a long-term factorial nutrient addition experiment in the Panama canal watershed has found that N, P, and K all limit aspects of plant growth (Kaspari *et al.*, 2008; S. J. Wright *et al.*, 2011), and that responses to N addition in western Panama vary among species (Adamek *et al.*, 2009), it seems likely that numerous interacting forces influence the uptake and subsequent sequestration of individual nutrients in wood. Nonetheless, the result that rock-derived macronutrients in wood

generally track the availability of these nutrients in the soil has implications for ecosystem and community processes.

Although soil nutrient concentrations were strong predictors of community mean wood nutrients at the plot level, wide variation in species mean nutrient concentrations in a given habitat suggest that species were unlikely to be excluded completely from a habitat based on nutrient allocation patterns, either physiologically or competitively. However, comparison of the slopes of wood vs. soil regression models indicates that basal area weighted community mean wood nutrient values (CWM) are more sensitive to soil nutrient variation than the unweighted average across species, providing evidence that tradeoffs related to nutrient allocation underlie shifts in species abundance across soil gradients. Alternatively, this pattern could occur if canopy trees differ systematically in wood nutrient allocation from smaller stature trees. In a Jamaican montane forest, Tanner, (1985) attributed the significantly lower nutrient concentrations of taller trees relative to short trees as a potential reason that tall trees were able to attain high biomass on nutrient impoverished soils. For 42 tree species in Bolivia, maximum tree height has been linked to wood anatomical traits including wood density, vessel diameter, and hydraulic conductance (Poorter, McDonald*, et al.*, 2010), which could have indirect effects on wood nutrient uptake and storage. Given that dominant canopy species disproportionately influence estimates of forest aboveground biomass (Slik *et al.*, 2013; Bastin *et al.*, 2015), efforts to quantify forest nutrient stocks in living biomass should prioritize the sampling of species with high basal area in a given community.

The strength of the relationship between species mean wood nutrient concentrations and soil nutrients were strongest for Ca and P, which were also the soil nutrients most closely associated with tree species distributions in lowland Panama (Condit *et al.*, 2013). This pattern

provides evidence that differences among species in the acquisition and use of Ca and P is partially responsible for their distribution across edaphically heterogeneous landscapes. Small within species sample size and high species turnover among sites prevented us from assessing how much of this response was explained by taxonomic or environmental controls. In Amazonian tree species, foliar P and Ca concentrations are more sensitive to environmental variation in soil availability than N and Mg concentrations (Fyllas *et al.*, 2009), and experimental nutrient addition in tropical forests show that both foliar and wood P concentrations respond more strongly to nutrient addition than do N concentrations (Harrington *et al.*, 2001; Ostertag, 2010; Schreeg *et al.*, 2014; Mo *et al.*, 2015). Wood Ca and P may also be more sensitive than other elements to soil conditions due to respective variation in the soil available nutrient pools, because the ranges of soil available Ca (115-fold) and P (90-fold) were vastly greater than the variation in Mg (38-fold), K (20-fold) and inorganic N (6-fold) among the ten plots sampled (Table 1). However, the heterogeneity in Ca and P in this region may provide a basis for the evolution of tradeoffs in Ca and P allocation strategies to optimize fitness in high versus low resource environments. Similarly, foliar P is the primary leaf trait differentiating tree species adapted to habitats differing widely in soil nutrient availability in Borneo (Baltzer & Thomas, 2010). Therefore, P allocation may be a key component of edaphic specialization niches in tropical regions worldwide. In regions with less spatial variation in soil nutrient availability than Borneo or Panama, weaker selection for tradeoffs in nutrient allocation might results in less variation among species and sites in wood nutrient concentrations.

Wood-leaf nutrient scaling

The scaling relationship between species mean wood and leaf tissues was significant for all nutrients for species examined along the Fortuna nutrient gradient, supporting findings of

previous meta analyses that have shown that N and P allocation is constrained across plant organs (Kerkhoff *et al.*, 2006; Ågren, 2008). However, our results differ from previous observations that wood and leaf nutrients concentrations are not closely correlated within angiosperm taxa (Pietsch *et al.*, 2014; Zanne *et al.*, 2015). Significant scaling between the PICs wood and leaf Ca, K, N and P indicate that the apparent functional coordination of nutrient allocation across organs is not simply a consequence of evolutionary history. While wood and leaf nutrients are correlated for all elements, the shape of scaling relationships differed among nutrients. Nonlinear scaling of wood and leaf nutrients for all elements except N indicated that leaf nutrients are more constrained than wood nutrients at high concentrations, perhaps because allocation of nutrients to woody repositories increases when nutrients are no longer limiting to photosynthesis. Dynamics of N storage may differ from other elements because plants generally store N as organic amino acids or proteins (Chapin *et al.*, 1990) and the storage of organic N may incur a substantial carbon cost relative storage of inorganic molecules (P Millard, 1988). In contrast, for P, which can be stored as inorganic phosphate in vacuoles (Sinclair & Vadez, 2002), the wood-leaf scaling exponent was ~2, meaning that, for example, a 10% increase in foliar P corresponds to a 20% increase in wood P. The apparent accumulation of P in wood at high foliar P concentrations may reflect the evolutionary importance of P storage reserves. Compared to other macronutrients, P is particularly immobile in soil solution and is spatially and temporally variable in its availability to plants (Lambers *et al.*, 2008). This indicates that trees may be under selection to allocate excess P to storage to mitigate P limitation when P demands of plant growth exceed P supply from soil.

Correlation of wood nutrients with life history parameters

We did not find support for the hypothesis from the WES that wood nutrient storage increases with wood density (Chave *et al.*, 2009). These results are in contrast to the findings of Martin *et al.*, 2014) in which wood N was positively correlated with wood density along this axis in tree species in the Panama Canal watershed, but corroborate the findings of Becker *et al.*, 2012), which found no relationship between wood N and wood density among tree species in Uganda. Given that wood and leaf nutrients were positively correlated, wood nutrients likely reflect whole plant allocation strategies or access to soil resources rather than active allocation of nutrients to wood storage reserves at the expense of other organs. In fact, wood P declined with increasing wood density in our montane forest, suggesting that low biomass P concentrations and dense wood are traits related to the survival of tropical tree species on low fertility habitats. While previous studies have reported that tree communities on low fertility soils have higher wood density (Muller-Landau, 2004; Chave et al., 2006) and lower wood P concentrations (Tanner, 1985) than communities on more fertile soils, this study is the first to our knowledge to test a functional relationship between these traits in tropical tree species. The significant relationship between the PICs of wood density and wood P indicate that this pattern is not solely a consequence of evolutionary history and might represent coordinated evolution of traits that facilitate survival on low resource habitats.

Radial variation in wood nutrient concentrations

Wood nutrients concentrations of the outermost 5 cm of the trunk strongly predicted nutrient concentrations of the adjacent inner segment for all nutrients, suggesting that radial variation in wood nutrients does not qualitatively influence patterns of interspecific or intersite variation in wood nutrients. Restranslocation of P from sapwood as it transitions to heartwood

appears to be a widespread among Panamanian trees, as wood P was lower in the inner annulus than the outer annulus for 76% of individuals cored. While this study did not evaluate differences between heartwood and sapwood explicitly, our results are consistent with (Meerts, 2002), which found heartwood concentrations were significantly lower than sapwood concentrations for 59 of 64 tree species. Radial variation in wood Ca, K, Mg, and N concentrations were more idiosyncratic across species. While Meerts, 2002), a dataset of predominately temperate species, found that wood N and K concentrations were higher in sapwood than heartwood for the majority of species, there was no consistent pattern across species or individuals for these elements in our study. Given that the increased remobilization efficiency of P compared to N from senesced leaves is used as a primary indicator that tropical forests are more P-limited than temperate forests (Vitousek, 1984), the increased retranslocation efficiency of P from sapwood relative to other elements may be evidence that the trees in our study were primarily P-limited. Because trees retain metabolically inactive wood in their stems, retranslocation of nutrients may be easier to quantify at the tree and species level in wood cores than in leaf litter, and should perhaps be utilized more often in studies of plant nutrient dynamics. Furthermore, if radial P translocation is greater in tropical forests than other ecosystems, it may exacerbate the allometric decline in whole plant N:P and C:P ratios (Elser *et al.*, 2010), which are used to calculate ecosystem nutrient stocks. As this study provides only a cursory evaluation into radial variation in wood nutrients, studies explicitly examining both the effect of continuous radial variation and heartwood/sapwood status on wood nutrients across species and sites are needed to improve our understanding of the function of wood nutrients.

Ecosystem implications of variation in wood nutrients

The marked variation in wood nutrient concentrations both among and within soil habitats observed here reinforces the idea that a trait-based approach using species-specific nutrient data could improve estimates of ecosystem processes in diverse tropical forests (Cornwell *et al.*, 2008). The scaling relationships of wood and leaf nutrients suggest that leaf nutrient values in global plant trait databases have potential to be used to assign wood nutrient content used in models predicting carbon turnover from woody biomass (Cornwell *et al.*, 2009). Given that tissue nutrient concentrations may be more important than wood anatomy and exogenous environmental factors in determining wood decomposition rates (Zanne *et al.,* 2015), the 8-fold range in community mean wood P concentrations observed here could translate to substantial differences in carbon residence time along fertility gradients. Not only are wood nutrient concentrations important for predicting dynamics of coarse woody debris, nonlinear scaling of species wood and leaf P concentrations suggest that trees store excess P in wood, which has implications for how tropical trees will respond to future global change scenarios. Understanding the extent to which P can be remobilized from woody tissues could improve predictions of how the growth of tropical trees will respond to the alleviation of other limiting factors via CO_2 fertilization and N deposition.

Conclusions

Wood stores more biomass than any other plant organ, and the fate of carbon and nutrients in the wood of tropical trees has particularly important implications of global biogeochemical cycles. Our study is among the first to demonstrate the enormous variability in wood nutrient concentrations among tropical tree species, and the strong relationship between community mean wood nutrients and soil resource availability. These results suggest that

allocation of limiting nutrients to woody biomass is an important functional characteristic influencing species distributions and biogeochemistry along edaphic gradients in tropical forests.

ACKNOWLEDGMENTS

The authors are grateful for funding the National Science Foundation Doctoral Dissertation Improvement Grant (DEB-1311379), Clark Research Support Grant, Tinker Summer Research Fellowship, and Program for Ecology, Evolution, and Conservation Summer Research Grant. We thank the Center for Tropical Forest Science for providing the census data for the 1-ha plots in the Panama Canal Watershed, and the Smithsonian Tropical Research Institute (STRI) for logistical support. We thank Carmen Velasquez, Fredy Miranda, and Robert Heineman for field assistance, Arturo Morris Valdes for identification of trees the Fortuna Forest Reserve, and Dayana Agudo and Paola Escobar for assisting with chemical analysis of wood at the STRI Soil Lab. We thank also Claire Baldeck, Adriana Corrales, and Jennifer Jones for comments that improved this manuscript.

LITERATURE CITED

Adamek M, Corre MD, Hölscher D. **2009**. Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, panama. *Journal of Tropical Ecology* **25**: 637-647.

Ågren GI. **2008**. Stoichiometry and nutrition of plant growth in natural communities. *Annual review of ecology, evolution, and systematics* **39**: 153-170.

Andersen KM, Corre MD, Turner BL, Dalling JW. **2010**. Plant–soil associations in a lower montane tropical forest: Physiological acclimation and herbivore-mediated responses to nitrogen addition. *Functional Ecology* **24**: 1171-1180.

Andersen KM, Endara MJ, Turner BL, Dalling JW. **2012**. Trait-based community assembly of understory palms along a soil nutrient gradient in a lower montane tropical forest. *Oecologia* **168**: 519-531.

Asner GP, Martin RE. **2011**. Canopy phylogenetic, chemical and spectral assembly in a lowland amazonian forest. *New Phytologist* **189**: 999-1012.

Baillie IC, Ashton PS, Anderson JAR, Fitzpatrick EA, Tinsley J, others. **1987**. Site characteristics and the distribution of tree species in mixed dipterocarp forest on tertiary sediments in central sarawak, malaysia. *Journal of Tropical Ecology* **3**: 201-220.

Bakker MA, Carreño-Rocabado G, Poorter L. **2011**. Leaf economics traits predict litter decomposition of tropical plants and differ among land use types. *Functional Ecology* **25**: 473-483.

Baldeck CA, Harms KE, Yavitt JB, John R, Turner BL, Valencia R, Navarrete H, Davies SJ, Chuyong GB, Kenfack D et al. 2013. Soil resources and topography shape local tree community structure in tropical forests. *Proceedings of the Royal Society of London B: Biological Sciences* **280**: 20122532.

Baltzer JL, Thomas SC. **2010**. A second dimension to the leaf economics spectrum predicts edaphic habitat association in a tropical forest. *PLoS One* **5**: e13163.

Baltzer JL, Thomas SC, Nilus R, Burslem DFR. **2005**. Edaphic specialization in tropical trees: Physiological correlates and responses to reciprocal transplantation. *Ecology* **86**: 3063-3077.

Baraloto C, Timothy Paine CE, Poorter L, Beauchene J, Bonal D, Domenach A-M, Hérault B, Patiño S, Roggy J-C, Chave J. **2010**. Decoupled leaf and stem economics in rain forest trees. *Ecology Letters* **13**: 1338-1347.

Bastin JF, Barbier N, Réjou-Méchain M, Fayolle A, Gourlet-Fleury S, Maniatis D, de Haulleville T, Baya F, Beeckman H, Beina D et al. 2015. Seeing central african forests through their largest trees. *Scientific reports* **5:** 13156.

Becker GS, Braun D, Gliniars R, Dalitz H. **2012**. Relations between wood variables and how they relate to tree size variables of tropical african tree species. *Trees* **26**: 1101-1112.

Bond WJ. **2010**. Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant and Soil* **334**: 47-60.

Cavelier J, Jaramillo M, Solis D, de León D. **1997**. Water balance and nutrient inputs in bulk precipitation in tropical montane cloud forest in panama. *Journal of Hydrology* **193**: 83-96.

Chapin FS. **1980**. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233-260.

Chapin FS, Schulze E-D, Mooney HA. **1990**. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* **21**: 423-447.

Chave J, Muller-Landau HC, Baker TR, Easdale TA, Steege Ht, Webb CO. 2006. Regional and phylogenetic variation of wood density across 2456 neotropical tree species. *Ecological Applications* **16**: 2356-2367.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. **2009**. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351-366.

Condit R, Engelbrecht BMJ, Pino D, Pérez R, Turner BL. **2013**. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences* **110**: 5064-5068.

Cornelissen J, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich D, Reich P, Ter Steege H, Morgan H, Van Der Heijden M. **2003**. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian journal of Botany* **51**: 335-380.

Cornwell WK, Cornelissen JHC, Allison SD, Bauhus J, Eggleton P, Preston CM, Scarff F, Weedon JT, Wirth C, Zanne AE. **2009**. Plant traits and wood fates across the globe: Rotted, burned, or consumed? *Global Change Biology* **15**: 2431-2449.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**: 1065-1071.

Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ. **2010**. Biological stoichiometry of plant production: Metabolism, scaling and ecological response to global change. *New Phytologist* **186**: 593-608.

Engelbrecht BMJ, Dalling JW, Pearson TRH, Wolf RL, Galvez DA, Koehler T, Tyree MT, Kursar TA. **2006**. Short dry spells in the wet season increase mortality of tropical pioneer seedlings. *Oecologia* **148**: 258-269.

Felsenstein J. **1985**. Phylogenies and the comparative method. *American Naturalist* **1**: 1-15.

Fine PVA, Mesones I, Coley PD. **2004**. Herbivores promote habitat specialization by trees in amazonian forests. *Science* **305**: 663-665.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JH. **2013**. Linking litter decomposition of above-and below-ground organs to plant–soil feedbacks worldwide. *Journal of Ecology* **101**: 943-952.

Fyllas NM, Patino S, Baker TR, Bielefeld Nardoto G, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna V, Mercado LM et al. 2009. Basin-wide variations in foliar

properties of amazonian forest: Phylogeny, soils and climate. *Biogeosciences* **6**: 2677-2708.

Garland T, Harvey PH, Ives AR. **1992**. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic biology* **41**: 18-32.

Gartlan JS, Newbery DM, Thomas DW, Waterman PG. **1986**. The influence of topography and soil phosphorus on the vegetation of korup forest reserve, cameroun. *Vegetatio* **65**: 131-148.

Han W, Fang J, Guo D, Zhang Y. **2005**. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in china. *New Phytologist* **168**: 377-385.

Han W, Fang J, Reich PB, Ian Woodward F, Wang Z. **2011**. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in china. *Ecology Letters* **14**: 788-796.

Harrington RA, Fownes JH, Vitousek PM. **2001**. Production and resource use efficiencies in n- and p-limited tropical forests: A comparison of responses to long-term fertilization. *Ecosystems* **4**: 646-657.

Hayes P, Turner BL, Lambers H, Laliberté E. **2014**. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology* **102**: 396-410.

John R, Dalling JW, Harms KE, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M et al. 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 864-869.

Jordan CF, Kline JR. **1977**. Transpiration of trees in a tropical rainforest. *Journal of Applied Ecology* **14**: 853-860.

Karla YP. 1998. Handbook of reference methods for plant analysis: CRC Press, Boca Raton, FL.

Kaspari M, Garcia MN, Harms KE, Santana M, Wright SJ, Yavitt JB. **2008**. Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters* **11**: 35-43.

Katabuchi M, Kurokawa H, Davies SJ, Tan S, Nakashizuka T. **2012**. Soil resource availability shapes community trait structure in a species-rich dipterocarp forest. *Journal of Ecology* **100**: 643-651.

Kattge J, Diaz S, Lavorel S, Prentice I, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright I. **2011**. Try–a global database of plant traits. *Global Change Biology* **17**: 2905-2935.

Kerkhoff Andrew J, Fagan William F, Elser James J, Enquist Brian J. **2006**. Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *The American Naturalist* **168**: E103-E122.

Kraft NJB, Valencia R, Ackerly DD. **2008**. Functional traits and niche-based tree community assembly in an amazonian forest. *Science* **322**: 580-582.

Lachenbruch B, Moore JR, Evans R. 2011. Radial variation in wood structure and function in woody plants, and hypotheses for its occurrence In: Meinzer FC, Lachenbruch B, Dawson, TE, eds. *Size- and age-related changes in tree structure and function*. Dordrecht, NL: Springer Science & Business Media, 121-164.

Lambers H, Raven JA, Shaver GR, Smith SE. **2008**. Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution* **23**: 95-103.

Legendre P. 2011. Lmodel2: Model II regression. R package version 1.7-0.

Marschner H. **1995**. Functions of mineral nutrients: Macronutrients. *Mineral nutrition of higher plants* **2**: 379-396.

Martin AR, Erickson DL, Kress WJ, Thomas SC. **2014**. Wood nitrogen concentrations in tropical trees: Phylogenetic patterns and ecological correlates. *New Phytologist* **204**: 484-495.

Mascaro J, Hughes RF, Schnitzer SA. **2012**. Novel forests maintain ecosystem processes after the decline of native tree species. *Ecological Monographs* **82**: 221-228.

Meerts P. **2002**. Mineral nutrient concentrations in sapwood and heartwood: A literature review. *Annals of Forest Science* **59**: 10.

Mehlich A. **1984**. Mehlich 3 soil test extractant: A modification of mehlich 2 extractant. *Communications in Soil Science & Plant Analysis* **15**: 1409-1416.

Millard P. **1988**. The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment* **11**: 1-8.

Mo Q, Zou B, Li Y, Chen Y, Zhang W, Mao R, Ding Y, Wang J, Lu X, Li X. **2015**. Response of plant nutrient stoichiometry to fertilization varied with plant tissues in a tropical forest. *Scientific reports* **5:** 14605.

Mooney HA, Gulmon SL. **1982**. Constraints on leaf structure and function in reference to herbivory. *BioScience* **32**: 198-206.

Muller-Landau HC. 2004. Interspecific and inter-site variation in wood specific gravity of tropical trees. *Biotropica* **36**: 20-32.

Ordoñez JC, Van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R. **2009**. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* **18**: 137-149.

Ostertag R. **2010**. Foliar nitrogen and phosphorus accumulation responses after fertilization: An example from nutrient-limited hawaiian forests. *Plant and Soil* **334**: 85-98.

Palmiotto PA, Davies SJ, Vogt KA, Ashton MS, Vogt DJ, Ashton PS. **2004**. Soil-related habitat specialization in dipterocarp rain forest tree species in borneo. *Journal of Ecology* **92**: 609-623.

Paradis E, Claude J, Strimmer K. **2004**. Ape: Analyses of phylogenetics and evolution in r language. *Bioinformatics* **20**: 289-290.

Phillips OL, Vargas PN, Monteagudo AL, Cruz AP, Zans M-EC, Sánchez WG, Yli-Halla M, Rose S. **2003**. Habitat association among amazonian tree species: A landscape-scale approach. *Journal of Ecology* **91**: 757-775.

Pietsch KA, Ogle K, Cornelissen JH, Cornwell WK, Bönisch G, Craine JM, Jackson BG, Kattge J, Peltzer DA, Penuelas J. **2014**. Global relationship of wood and leaf litter decomposability: The role of functional traits within and across plant organs. *Global Ecology and Biogeography* **23**: 1046-1057.

Poorter L, Bongers F. **2006**. Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology* **87**: 1733-1743.

Poorter L, Kitajima K. **2007**. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology* **88**: 1000-1011.

Poorter L, McDonald I, Alarcón A, Fichtler E, Licona JC, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U. **2010**. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* **185**: 481-492.

Pyke CR, Condit R, Aguilar S, Lao S. **2001**. Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science* **12**: 553-566.

Quesada CA, Lloyd J, Schwarz M, Baker TR, Phillips OL, Patiño S, Czimczik C, Hodnett MG, Herrera R, Arneth A et al. 2009. Regional and large-scale patterns in amazon forest structure and function are mediated by variations in soil physical and chemical properties. *Biogeosciences Discussions* **6**: 3993-4057.

Reich PB, Oleksyn J. **2004**. Global patterns of plant leaf n and p in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 11001-11006.

Rozendaal D, Hurtado V, Poorter L. **2006**. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology* **20**: 207-216.

Santiago LS. **2007**. Extending the leaf economics spectrum to decomposition: Evidence from a tropical forest. *Ecology* **88**: 1126-1131.

Schreeg LA, Santiago LS, Wright SJ, Turner BL. **2014**. Stem, root, and older leaf n:P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology* **95**: 2062-2068.

Sellin A. **1994**. Sapwood-heartwood proportion related to tree diameter, age, and growth rate in picea abies. *Canadian Journal of Forest Research* **24**: 1022-1028.

Sinclair TR, Vadez V. **2002**. Physiological traits for crop yield improvement in low n and p environments. *Plant and Soil* **245**: 1-15.

Slik JW, Paoli G, McGuire K, Amaral I, Barroso J, Bastian M, Blanc L, Bongers F, Boundja P, Clark C et al. 2013. Large trees drive forest aboveground biomass variation in moist lowland forests across the tropics. *Global Ecology and Biogeography* **22**: 1261-1271.

Stanley WG, Montagnini F. **1999**. Biomass and nutrient accumulation in pure and mixed plantations of indigenous tree species grown on poor soils in the humid tropics of costa rica. *Forest Ecology and Management* **113**: 91-103.

Tanner EVJ. **1977**. Four montane rain forests of jamaica: A quantitative characterization of the floristics, the soils and the foliar mineral levels, and a discussion of the interrelations. *The Journal of Ecology* **65**: 883-918.

Tanner EVJ. **1985**. Jamaican montane forests: Nutrient capital and cost of growth. *The Journal of Ecology* **73**: 553-568.

Tanner EVJ, Vitousek PM, Cuevas E. **1998**. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**: 10-22.

Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. **2007**. Controls over foliar n:P ratios in tropical rain forests. *Ecology* **88**: 107-118.

Turner BL, Engelbrecht BM. **2011**. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**: 297-315.

Turner BL, Romero TE. **2009**. Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal* **73**: 1972.

Vitousek PM. **1984**. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **65**: 285-298.

Vitousek PM, Matson PA, Turner DR. **1988**. Elevational and age gradients in hawaiian montane rainforest: Foliar and soil nutrients. *Oecologia* **77**: 565-570.

Wang D, Bormann FH, Lugo AE, Bowden RD. **1991**. Comparison of nutrient-use efficiency and biomass production in five tropical tree taxa. *Forest Ecology and Management* **46**: 1-21.

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, Van Der Putten WH, Wall DH. **2004**. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629-1633.

Webb CO, Ackerly DD, Kembel SW. **2008**. Phylocom: Software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098-2100.

Webb CO, Donoghue MJ. **2005**. Phylomatic: Tree assembly for applied phylogenetics. *Molecular Ecology Notes* **5**: 181-183.

Weedon JT, Cornwell WK, Cornelissen JHC, Zanne AE, Wirth C, Coomes DA. **2009**. Global meta-analysis of wood decomposition rates: A role for trait variation among tree species? *Ecology Letters* **12**: 45-56.

Wikström N, Savolainen V, Chase MW. **2001**. Evolution of the angiosperms: Calibrating the family tree. *Proceedings of the Royal Society of London B: Biological Sciences* **268**: 2211-2220.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE et al. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**: 1616-1625.

Würth MK, Pelaez-Riedl S, Wright SJ, Körner C. **2005**. Non-structural carbohydrate pools in a tropical forest. *Oecologia* **143**: 11-24.

Zanne, AE, Oberle, B, Dunham, KM, Milo, AM, Walton, ML, Young, DF. 2015. A deteriorating state of affairs: How endogenous and exogenous factors determine plant decay rates. *Journal of Ecology* **103**: 1421-1431.

Table 3.1 Site location, environmental characteristics, and species richness of 10 1-ha Panamanian forest plots near which wood core samples were collected.

	Montane Forest: Fortuna						Lowland Forest: Panama Canal Watershed			
Environmental Variables	Chorro A	Honda A	Samudio	Palo Seco	Hornito	Alto Frio	P25	P06	P13	P24
[1]Protected area	Fortuna	Fortuna	Fortuna	Palo Seco	Fortuna	Fortuna	ACP	Soberanía	BCNM	Soberanía
Latitude (N)	8°45'42"	8°43'3"	8°43'52"	8°46'43"	8°40'26"	8°39'15"	9°4'42"	9°9'23"	9°11'16"	9°7'25"
Longitude (W)	82°14'32"	82°14'22"	82°14'53"	82°11'53"	82°12'15''	82°12'54"	79°47'56"	79°44'39"	79°49'16"	79°40'36"
Elevation (m)	1100	1155	1215	878	1330	1100	110	30	55	50
[2]Annual Rainfall (mm yr^{-1})	5500	6200	4800	6200	5100	4600+	2100	2300	2600	2200
Dry Season Rainfall (mm mo^{-1})	351	381	215	445	203	91	124	149	197	131
[3]***Soil Properties***										
Geological Substrate	Rhyolite	Rhyolite	Andesite	Andesite	Dacite	Dacite	Rhyolite	Marine Sediment (Gatuncillo)	Marine Sediment (Caimito)	Agglomerate
Bulk Density (g cm^{-3})	0.13	0.29	0.39	0.41	0.25	0.66	0.87	1.05	0.67	0.59
pH	3.67	3.58	4.18	4.37	5.03	5.62	4.61	4.36	6.39	6.36
Inorganic N (mg cm^{-3})	1.01	3.44	1.8	1.6	3	6.32	2.54	2.46	3.07	3.01
Resin P (mg cm^{-3})	0.08	0.22	0.42	0.43	2.22	1.39	0.18	1.28	5.65	7.75
Mehlich Ca (mg cm^{-3})	97	82	249	135	1358	3388	32	379	3706	1662
Mehlich K (mg cm^{-3})	19	11	64	31	96	48	32	30	211	207
Mehlich Mg (mg cm^{-3})	19	20	53	40	254	551	67	117	737	539
[4]***Forest Structure***										
Species Richness	72	135	125	167	139	83	84	78	60	60
Basal Area (m^2 ha^{-1})	32	44	35	32	55	43	20	19	25	31
Stem Density (stems ha^{-1})	1042	813	805	681	715	1050	302	484	429	355
Species Sampling										
Species Cored	13	22	19	18	14	17	11	9	9	7
BA represented by cored trees	71%	68%	45%	30%	72%	54%	41%	28%	64%	21%

[1]Protected areas: Fortuna Forest Reserve (Fortuna), Palo Seco Forest Protectorate (Palo Seco), Panama Canal Authority (ACP), Soberanía National Park (Soberanía), and Barro Colorado Island Nature Monument (BCNM).

Table 3.2 Summary statistics of community-weighted mean (CWM) and species wood nutrient concentrations for 106 tree species sampled across six lowland and four montane forest plots.

	Community Weighted Means (μg g^{-1})			Species Means (μg g^{-1})		
Nutrient	Mean	Min	Max	Mean	Min	Max
Ca	2038	823	5692	2082	271	12613
K	1547	653	2935	1622	157	5771
Mg	526	320	807	492	61	3123
N	2603	1857	3454	2557	1300	5800
P	135	44	339	111	19	668
N:P	34	14	59	35	4	181
C:N	203	148	267	209	80	381
C:P	7276	2175	14881	7510	729	50400

Table 3.3 Major axis regression model fits of the scaling relationship between observed species mean wood and leaf nutrient concentrations (log(leaf) ~ log(*a*) + log(wood)**b*) and the phylogenetically independent contrasts (PICs) of species mean wood and leaf concentrations (PIC of log leaf ~ PIC of log wood**b*) for 58 tree species sampled at Fortuna Forest Reserve.

			Intercept: log (a)			Slope: b				
Nutrient	*Model*	*N*	*mean*	*2.5*	*97.5*	*mean*	*2.5*	*97.5*	r^2	*P*
Ca	Observed	58	-10.61	-5.35	1.36	1.93	1.36	3.00	0.33	< 0.001
	PIC	56				1.40	0.99	2.10	0.36	< 0.001
K	Observed	58	-11.03	-22.30	-5.33	1.97	1.36	3.19	0.30	< 0.001
	PIC	56				1.92	1.24	3.50	0.24	< 0.001
Mg	Observed	58	-17.72	-45.64	-8.76	2.93	1.82	6.39	0.18	< 0.001
	PIC	56				5.61	1.95	-8.14	0.03	0.223
N	Observed	58	-4.47	-11.45	-0.35	1.25	0.83	1.95	0.31	< 0.001
	PIC	56				1.01	0.84	1.23	0.49	< 0.001
P	Observed	58	-10.03	-17.42	-5.99	2.10	1.52	3.17	0.36	< 0.001
	PIC	56				2.22	1.85	2.67	0.54	< 0.001

Table 3.4 Major axis regression model fits of pairwise combinations of log transformed species mean wood nutrient (Ca, K, Mg, N, and P; μg g^{-1}) and wood density (WD; g cm^{-3}) measured in 76 tree species from six forest sites at Fortuna (*df* = 74).

		Intercept			Slope				
x variable	y variable	mean	2.5	97.5	mean	2.5	97.5	r^2	P^1
WD	Ca	-11.2	32.9	0.4	31.8	11.7	-44.5	0.02	0.249
	K	21.2	12.9	-22.5	-24.1	51.5	-9.7	0.02	0.179
	Mg	18.7	11.8	-96.1	-22.2	176.2	-10.4	0.04	0.081
	N	16.8	10.8	-2.1	-15.7	17.1	-5.3	0.01	0.302
	P	10.9	8.2	25.4	-11.2	-36.2	-6.6	0.10	0.005*
Ca	K	2.21	-2.75	5.22	0.70	0.29	1.39	0.10	0.006*
	Mg	-2.73	-21.42	2.53	1.18	0.46	3.78	0.07	0.024*
	N	5.90	4.85	6.88	0.26	0.12	0.41	0.16	< 0.001**
	P	-0.03	-3.60	2.44	0.62	0.28	1.12	0.12	0.002**
K	Mg	-5.86	-23.11	-0.34	1.60	0.84	3.97	0.11	0.005*
	N	5.42	4.15	6.58	0.32	0.16	0.50	0.18	< 0.001**
	P	-2.10	-4.93	-0.06	0.90	0.62	1.29	0.30	< 0.001**
Mg	N	6.65	5.82	7.45	0.19	0.06	0.34	0.10	0.007*
	P	0.61	-1.14	1.99	0.66	0.42	0.96	0.26	< 0.001**
N	P	-11.72	-17.61	-8.02	2.08	1.60	2.84	0.41	< 0.001**

[1]P values of marked with "*" where significant at $P < 0.05$ and "**" where significant correcting for multiple comparisons ($P < 0.0033$).

Table 3.5 Summary of log-log linear regression models testing the relationship between community weighted mean (CWM) wood nutrient concentrations and soil nutrient availability and species mean wood nutrient concentrations and soil nutrient availability.

	log CWM Wood vs. log Soil				log Species Mean Wood vs. log Soil			
Nutrient	Intercept	Slope	r^2	*P*	Intercept	Slope	r^2	*P*
Ca	5.94	0.25	0.49	0.014	6.12	0.21	0.19	< 0.001
K	5.87	0.36	0.58	0.006	6.52	0.18	0.06	0.003
Mg	5.31	0.19	0.64	0.003	5.56	0.07	0.01	0.141
N	7.75	0.100	0.00	0.516	7.75	0.03	0.00	0.564
P	4.76	0.40	0.71	0.001	4.59	0.28	0.31	< 0.001

Table 3.6 Major axis regression model fits of pairwise combinations of the phylogenetically independent contrasts (PIC) of species mean wood nutrient (Ca, K, Mg, N, and P; μg g^{-1}) and wood density (WD; g cm^{-3}) measured in 76 tree species from six forest sites at Fortuna (*df* = 74).

		Slope				
x variable	y variable	mean	2.5	97.5	r^2	P^1
WD	Ca	99.4	16.8	-25.3	0.00	0.686
	K	-19.5	90.8	-8.8	0.04	0.105
	Mg	-22.7	-1699.8	-11.4	0.05	0.047*
	N	-17.9	15.9	-5.7	0.01	0.349
	P	-12.2	-21.9	-8.5	0.22	< 0.001**
Ca	K	0.51	0.16	0.99	0.10	0.006*
	Mg	1.34	0.62	3.64	0.10	0.006*
	N	0.24	0.12	0.38	0.17	< 0.001**
	P	1.47	0.91	2.66	0.19	< 0.001**
K	Mg	2.69	1.36	13.09	0.08	0.016*
	N	0.24	0.04	0.46	0.07	0.020*
	P	2.25	1.47	4.07	0.20	< 0.001**
Mg	N	0.25	0.15	0.36	0.24	< 0.001**
	P	1.16	0.77	1.80	0.26	< 0.001**
N	P	3.23	2.59	4.23	0.49	< 0.001**

[1]P values of marked with "*" where significant where $P < 0.05$ and "**" where significant correcting for multiple comparisons ($P < 0.0033$).

Table 3.7 Major axis regression model fits of the scaling relationship between nutrient concentrations in the outermost 5 cm annulus of wood vs. the adjacent 5-10 cm annulus of wood cores (log(inner) ~ log(*a*) + log(outer)**b*) for 110 trees in Panama.

		Intercept: log(a)			Slope: b				
Nutrient	*N*	*mean*	*2.5*	*97.5*	*mean*	*2.5*	*97.5*	r^2	*P*
Ca	110	0.33	-0.38	0.98	0.97	0.88	1.07	0.75	< 0.001
K	110	-2.88	-4.24	-1.68	1.42	1.24	1.61	0.53	< 0.001
Mg	110	0.26	-0.26	0.73	0.97	0.88	1.06	0.78	< 0.001
N	110	-1.49	-2.41	-0.65	1.19	1.08	1.31	0.75	< 0.001
P	110	-1.56	-2.25	-0.95	1.26	1.12	1.42	0.61	< 0.001

Figure 3.1 Locations of ten permanent 1-ha forest plots in Panama where we sampled wood. Leaves were also collected from the six Fortuna plots. Panama Canal Watershed sites are seasonally moist lowland forest located < 110 m asl. Fortuna sites are wet lower montane forest located 800-1300 m asl. Plots are grouped by low, medium, and high fertility based on soil chemical variables and geological parent material (see Table 3.1).

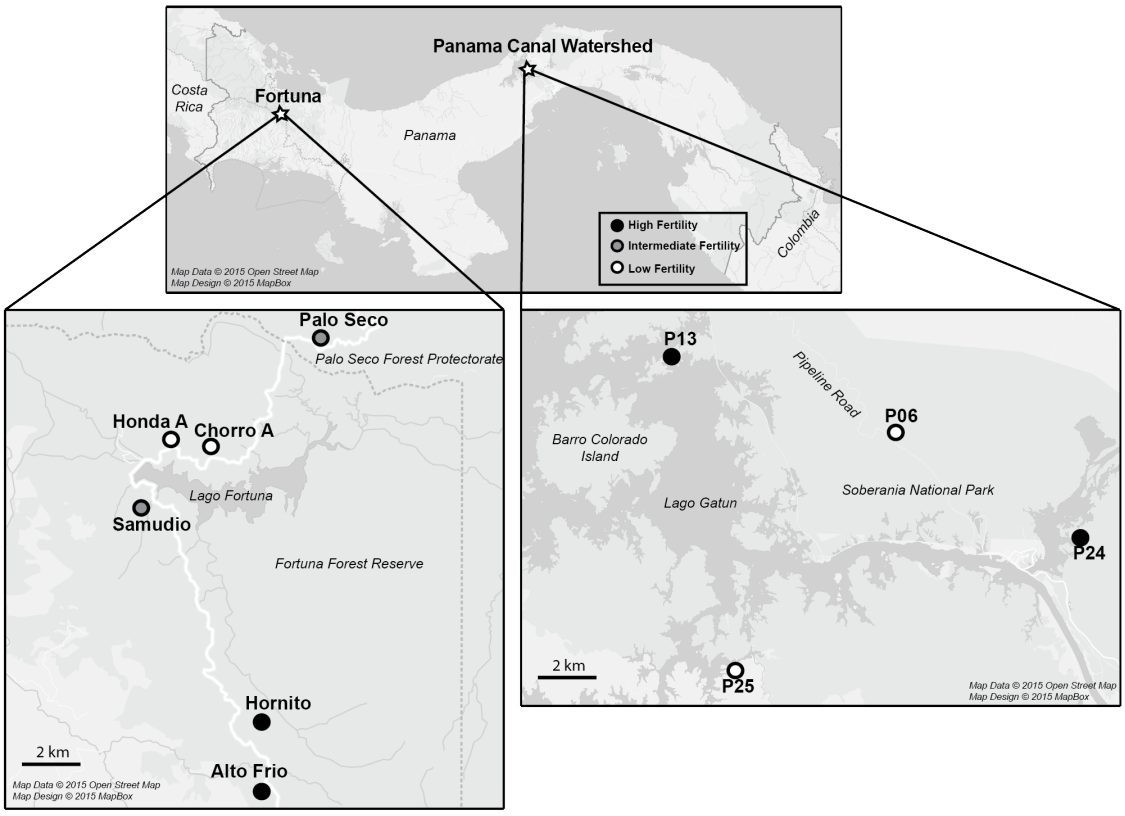


Figure 3.2 Wood nutrient concentrations (of the outer 5 cm of sapwood) vs. soil nutrient availability in four lowland (open circles) and six montane (closed circles) forest plots Panama. Soil nutrients were measured in the top 10 cm of soil and are expressed in volumetric units (mg cm^{-3}). Lines of log-log linear regression models are presented where the relationship between wood and soil nutrients is significant. (a-e) Community weighted mean (CWM) wood nutrient concentrations of all species sampled in each plot. Y bars = one basal area-weighted standard error. (f-j) Species mean wood nutrient concentrations plotted as a function of plot-level soil nutrient concentrations. Axes are plotted on log scales.

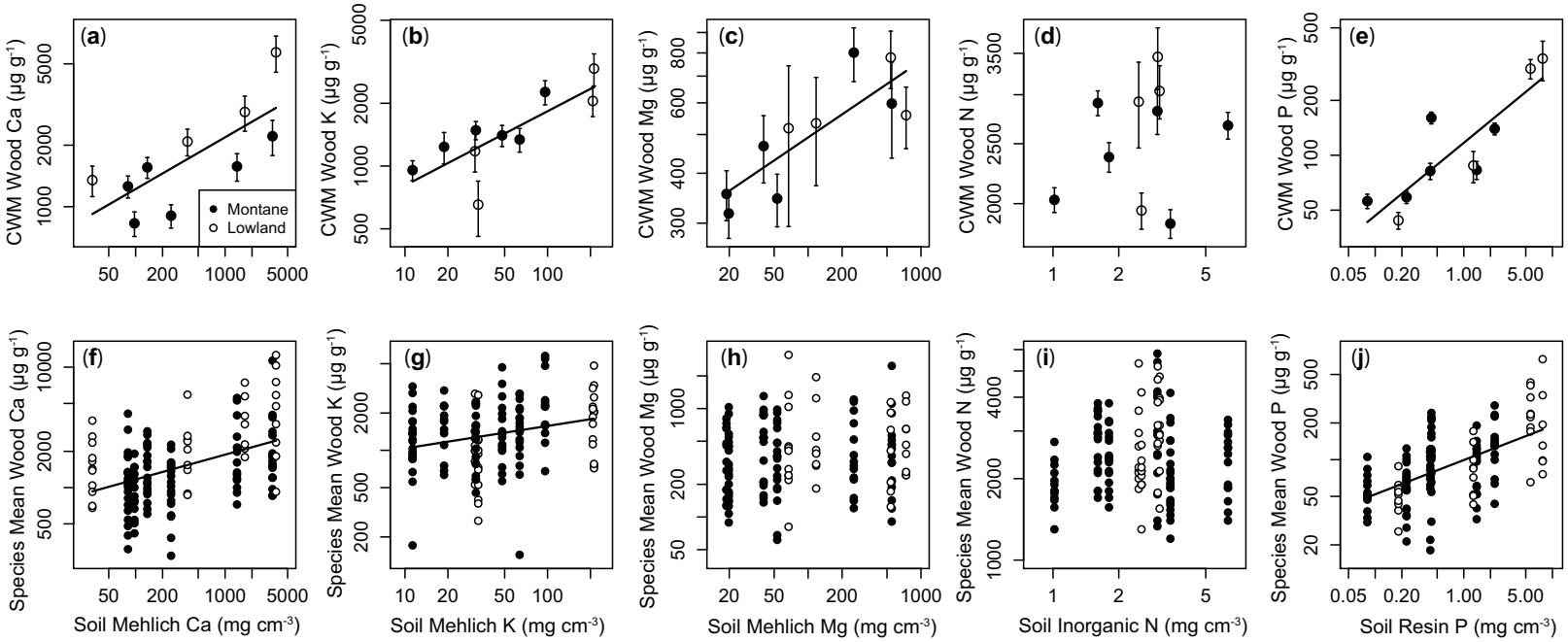


Figure 3.3 Scaling relationship of species mean leaf and wood nutrient concentrations for 58 tree species sampled in western Panama. Lines represent major axis regression model fits. Scaling relationships were fit for log-transformed observed species mean wood and leaf nutrient concentrations (a-e; closed circles) and for the phylogenetically independent contrasts (PIC) of logged species mean values (f-j; open circles). Wood nutrients were analyzed for the outermost 5 cm annulus of sapwood.

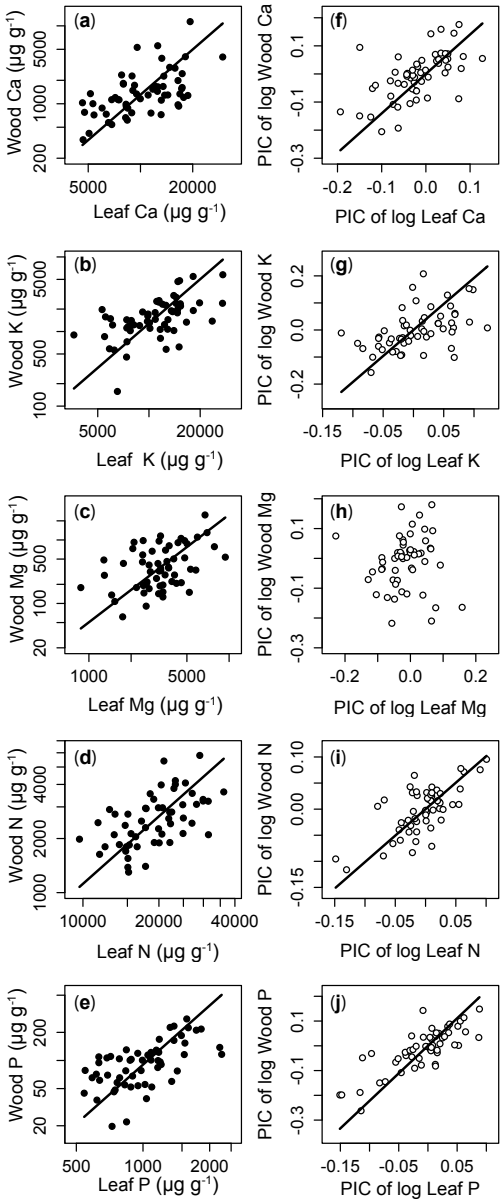


Figure 3.4 Scaling relationship of nutrient concentration in "outer" 5 cm of annulus of wood and adjacent "inner" 5-10 cm annulus for 110 trees cored in Panama. Black lines represent major axis (MA) regression model fits of the scaling relationship between nutrient concentrations in inner and outer (log(inner) ~ log(*a*) + log(outer)**b*). Blue dashed lines represent a 1:1 relationship.

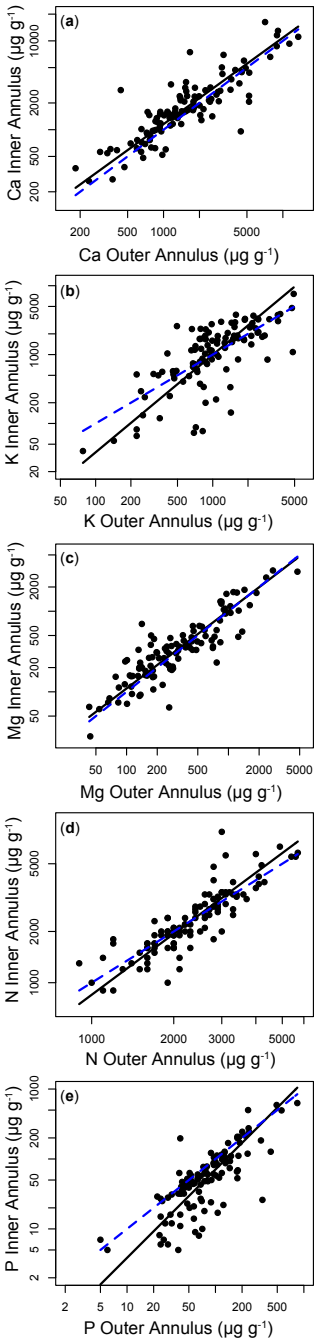
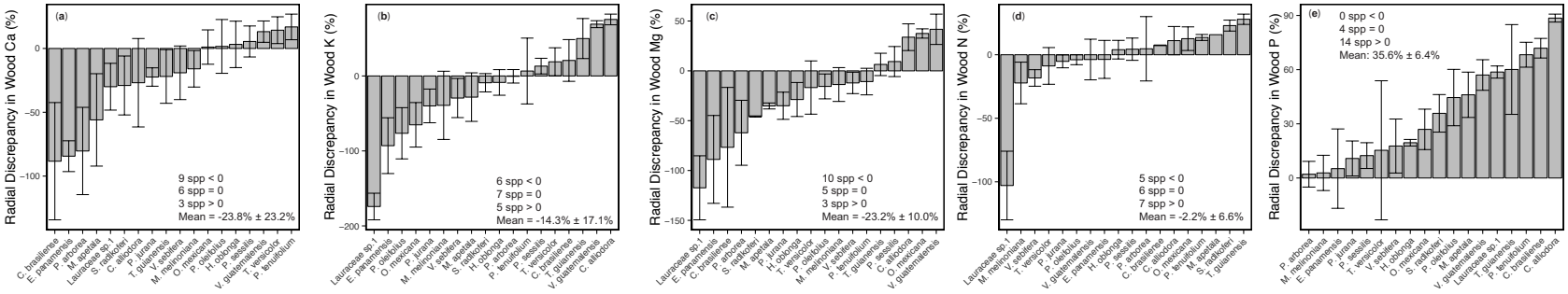


Figure 3.5 Species mean radial discrepancy in Ca, K, Mg, P, and N concentrations (a-e) between the outermost annulus of the stem (0-5 cm in depth) and the adjacent inner annulus (5-10 cm in depth) for 18 tree species in Panama. Radial discrepancy was calculated as (outer– inner)/outer * 100. Species mean values +/- standard error are reported on each panel



CHAPTER 4

EVALUATING THE INFLUENCE OF SPECIES TRAITS AND SOIL RESOURCE AVAILABILITY ON MULTIPLE STEM FREQUENCY IN PANAMA

ABSTRACT

Resprouting is a critical component of tree species life history that is associated with low growth rates, mortality rates, and carbohydrate storage. However, few studies have considered the influence of fertility on patterns of vegetative regrowth in tropical forests. We hypothesized that if resprouting is costly in terms of carbon and nutrients then the frequency of multi-stemmed trees (a potential indicator of resprouting history) should be lower on infertile soils where soil nutrient supply and plant nutrient reserves are insufficient to enable resprouting after damage. We used long term inventory data from the Barro Colorado Island (BCI) 50-ha forest dynamics plot in Panama to show that multi-stemmed trees are more likely to resprout during their lifetime than single-stemmed trees, and that multiple stem frequency has a strong taxonomic signal for adult trees. To test how multiple stem frequency co-varies with demographic rates and nutrient allocation strategies, we compiled two functional trait datasets including 71 species from lowland forest on BCI and 43 species from montane forest in western Panama. We then assessed environmental correlates of multiple stem frequency in 37 1-ha lowland plots in the Panama Canal watershed and 12 1-ha montane plots in western Panama, which span a regional soil fertility and rainfall gradient. The frequency of multi-stemmed individuals for a given species was not correlated with species demographic rates or wood density in either functional traits dataset, indicating that resprouting is not a 'persistence' trait in Panamanian tree species.

Multiple stem frequency of tree species co-occurring on BCI was weakly but significantly correlated to regional soil phosphorus associations but unrelated to foliar nutrient concentrations. Multiple stems frequency was significantly correlated with nutrient allocation traits, including foliar phosphorus and wood nitrogen and phosphorus, among species sampled across a fertility gradient in western Panama. At the community level, the proportion of woody stems > 10 cm diameter at beast height in a forest with at least one multiple stem increased with soil fertility, as represented by principal component axes, and with soil available phosphorus in particular. Together, these results suggest that soil nutrient availability facilitates resprouting by relieving nutrient limitation to vegetative growth. This process is a novel mechanism by which soil phosphorus availability influences tree recruitment and forest dynamics. Future experiments are needed to understand whether soil fertility mediates resprouting frequency through nutrient supply directly or through the formation of wood nutrient reserves.

INTRODUCTION

In forest ecosystems, the life of a tree is seldom the unobstructed path of a single stem from seedling to canopy. Large-scale disturbances such as hurricanes or fire and smaller-scale disturbances such as tree falls, herbivory, or disease may result in damage to a tree's principal stem that necessitates the formation of a secondary stem via resprouting. In North American forests, resprouting is so common in the understory that 58-79% of seedlings < 5 cm tall have resprouted at least once (Liming & Johnston, 1944; Merz & Boyce, 1956; Ward, 1966), and nearly all angiosperms retain the ability to resprout as saplings (Del Tredici, 2001).

Despite the ubiquity of resprouting, tree species differ widely in the form and frequency of their resprouting abilities, especially as plants reach reproductive maturity. In ecosystems where disturbance regimes are severe, such as frequently burned Mediterranean shrublands, the

distinction between non-sprouting and sprouting species is essentially binary, with plants that are completely destroyed from fire classified as "seeders" and plants that recover from fire classified as "sprouters" (Keeley, 1977). As the disturbance regime in an ecosystem becomes less severe, the anatomical form of resprouting shifts from "basal resprouting" where new stems are created from the collar of trees to "axillary resprouting," which may occur from buds in the stem or branches (Bellingham & Sparrow, 2000), and species tend to vary continuously rather than discretely in resprouting frequency (Vesk & Westoby, 2004). For instance, in tropical forests, where tree falls are the primary cause of disturbance, nearly all species have the ability to produce resprout as juveniles; however, sapling clipping experiments in Panama (Guariguata, 1998) and Bolivia (Poorter, Kitajima*, et al.*, 2010) found that species differ substantially in the ability to recover from damage, even within the same genus (Lasso *et al.*, 2009). This variation suggests that resprouting can be viewed as a functional trait in woody plants, with important implications for plant community assembly (Clarke *et al.*, 2010; Clarke *et al.*, 2013).

As a functional trait, resprouting is viewed as a persistence strategy (Bond & Midgley, 2001), meaning that resprouting requires an investment of resources towards future survival at the expense of upward growth and reproduction. Therefore, resprouting may partially underlie growth–mortality tradeoffs and should be correlated with other persistence traits such as wood density (Chave *et al.*, 2009; Wright *et al.*, 2010), non-structural carbohydrate reserves (Kobe, 1997; Poorter & Kitajima, 2007), and shade tolerance (Kitajima, 1994; Walters & Reich, 1996). However, evidence that resprouting is associated with the slow-growth is mixed. Results from a clipping experiment in Bolivia support the persistence hypothesis, finding that slow-growing, shade tolerant saplings with relatively high wood density and carbohydrate storage were more likely to survive experimental damage than trees with low wood density and high growth rates

(Poorter, Kitajima*, et al.*, 2010). In contrast, species resprouting frequency was unrelated to growth rate or light requirements for regeneration over 15 years of forest dynamics monitoring on Barro Colorado Island (BCI), Panama (Paciorek *et al.*, 2000), following artificial gap formation in southeastern United States (Dietze & Clark, 2008), or after forest clear-cutting in Japan (Shibata *et al.*, 2014). Furthermore, high wood density did not confer tolerance to trees damaged by logging in the Amazon (Shenkin *et al.*, 2015), and the resprouting ability in juvenile trees declined with increasing wood density among Japanese species (Shibata *et al.*, 2014). In light of mounting evidence that resprouting does fit squarely within the description of a "persistence strategy", the ecological correlates of tree species resprouting merit re-examination.

Curiously, the importance of soil nutrient availability and plant nutrient storage has been somewhat overlooked with respect to tree resprouting abilities (Clarke *et al.*, 2013), despite the fact that nitrogen (N) or phosphorus (P), either alone or in combination, limit productivity in nearly all terrestrial ecosystems (Elser *et al.*, 2007). While many studies have evaluated the relationship between tree species resprouting and mobile carbon reserves (Sakai & Sakai, 1998; Poorter, *et al.*, 2010), remobilization of N and P from foliar and woody tissues must presumably also occur during resprouting. Phosphorus limitation in particular might impede resprouting in tropical trees, which typically on strongly weathered soils that are low in P (Walker & Syers, 1976; Vitousek & Sanford, 1986). Soil P not only limits tree growth in many tropical forests (Vitousek, 1984), but also influences species distributions at regional scales (Condit *et al.*, 2013). Given that P accumulates in foliar and woody tissues in nutrient addition experiments (Harrington *et al.*, 2001; Ostertag, 2010; Schreeg *et al.*, 2014), trees on high fertility soils likely maintain larger P reserves than trees on low fertility soils, despite the fact that trees on low fertility soils have higher P reabsorption efficiency (Kitayama *et al.*, 2004; Hayes *et al.*, 2014).

Furthermore, tree species associated with high fertility soils have higher nutrient concentrations in plant biomass than species adapted to low fertility soils (Tanner, 1977; Andersen *et al.*, 2012; Katabuchi *et al.*, 2012; Dalling et al., 2016), indicating that shifts in species composition across soil gradients could exacerbate variation in nutrient reserves. Therefore, if resprouting depends partially on the size of nutrient reserves, then resprouting should increase with both the availability of nutrients in the soil and the concentrations of nutrients in plant biomass. High interspecific variation in foliar (Townsend *et al.*, 2007; Fyllas *et al.*, 2009) and woody (Heineman *et al.,* 2016) nutrient concentrations among co-occurring species on both high and low fertility soils suggests that formation and use of limiting nutrient reserves for resprouting should also very markedly among species in the same community.

The presence of secondary stems, or multiple stems, is commonly included in tropical forest plot census data (Condit, 1998) and can therefore be leveraged to investigate ecological correlates of resprouting over environmental gradients. Because trees can create multiple stems in the absence of damage to the apical meristem, multiple stems are not necessarily the result of a disturbance-induced resprouting event, nor is it true that trees without multiple stems in a given plot census are incapable of resprouting. However, because multiple stems require growth via non-apical meristem, the presence of one or more secondary stems indicates that a tree has the physiological potential to create resprouts in response to damage. Furthermore, on the 50 ha forest dynamics plot on BCI, where the incidence of both multiple stems and tree resprouting are directly measured in long term census data, trees with multiple stems had a 50% higher probability of resprouting than trees without multiple stems (Paciorek *et al.*, 2000), indicating that multiple stem frequency may be a valuable proxy of community and species level resprouting patterns in neotropical trees.

In this study, we use long term census data from BCI to assess whether (1) the presence of multiple stems predicts future resprouting ability and (2) multiple stem frequency is a trait constrained by taxonomy in tropical tree communities. We also evaluate how species frequency of multiple stemmed individuals co-varies with demographic and nutrient allocation traits for 114 tree species sampled in a large scale forest plot in lowland Panama and across a network of 1-ha plots in montane western Panama. We present plot level frequency of multiple-stemmed trees from 49 1-ha plots in lowland and lower montane forest sites in Panama spanning regional rainfall and soil fertility gradients. We hypothesized that if the creation of multiple stems occurs as a persistence strategy at the expense of principal stem growth, then multiple stem frequency would be inversely related to growth rate and mortality rates at the species level and communities on low fertility soils should have more multi-stemmed individuals than communities on high fertility soils. Alternatively, if the ability to create multiple stems depends on the nutrient reserves of a tree, then species resprouting frequency should increase with species wood and leaf nutrient concentrations, and the frequency of trees in a given plot with at least one multiple stem should increase with soil nutrient availability.

MATERIALS AND METHODS

Study site

The ecological correlates of multiple stem frequency in tropical forests were evaluated for two networks of permanent forest plots in Panama, including one 50-ha plot and 37 1-ha plots in the Panama Canal watershed and 12 1-ha plots in and around the Fortuna Forest Reserve in western Panama (Figure 4.1). BCI is a 1500-ha island that was isolated by the formation of the Panama Canal, and the 50-ha plot was established on the central plateau of BCI in 1980. The 1-ha plots in the Panama Canal watershed were established between 1994-2007 by the Center for

Tropical Forest Science (Pyke *et al.*, 2001; Turner & Engelbrecht, 2011; Condit *et al.*, 2013). This region consists of semi-deciduous, seasonally moist forest, receiving 2100-2600 mm of annual rainfall and with a mean annual temperature of 27°C (Pyke *et al.*, 2001). In the dry season, dry spells during which evapotranspiration exceeds rainfall for more than 10 days occur at least every other year (Engelbrecht *et al.*, 2006). There is considerable spatial variation in the severity of the dry season across the isthmus (Table 4.5), which strongly influences the distribution of tree species in Panama (Engelbrecht *et al.*, 2007). In addition, the complex geology of the area underlies striking variation among plots in soil nutrient availability (Table 4.5), especially soil available P (Turner & Engelbrecht, 2011), which, along with rainfall, influences tree species distributions patterns across the isthmus (Condit *et al.*, 2013). Successional age of each plot was classified as old growth, mature secondary, or young secondary by Pyke *et al.*, (2001). On BCI and the majority of 1-ha plots, all trees ≥ 1 cm diameter at breast height (1.3 m; DBH) were measured, tagged, and identified to species according to Condit, (1998). A handful of 1-ha plots had a minimum measurement size of 10 cm, but in these cases multiple stems > 1 cm were still measured. In cases where a tree had more than one stem ≥ 1 cm DBH, the largest stem was denoted as the "main stem" and smaller stems were measured and denoted as "secondary stems". While there are > 50 CTFS 1-ha plots in Panama, this analysis includes only those with available soil nutrient data, most of which have been published previously (Turner, 2010; Turner & Engelbrecht, 2011; Condit *et al.*, 2013).

The Fortuna plots were established between 2003-2008 by the University of Illinois and the Smithsonian Tropical Research Institute within the Fortuna Forest Reserve (19,500 ha) and the adjacent Palo Seco Forest Protectorate (125,000 ha), henceforth Fortuna, in western Panama (Figure 4.1). This region encompasses old growth, lower montane forest, ranging between 700

and 1500 m asl, with mean annual temperatures varying between 19 and 23°C (Cavelier *et al.*, 1997). There is strong interannual and spatial variability in precipitation among study sites, with annual rainfall ranging from 4000 to 9000 mm per year. A distinct dry season occurs from January to April, but evapotranspiration does not exceed rainfall during this period (Cavelier *et al.*, 1997). The strength of the dry season varies considerably among sites (Table 4.5), with plots on the Caribbean side of the Continental Divide having less distinct dry season (Prada *et al., in review*). Tree falls are likely the primary cause of understory disturbance, although there is evidence that episodic storm events cause severe canopy disturbance at Fortuna (Heineman *et al.*, 2015). There is also enormous variation in soil nutrient availability among plots at Fortuna (Table 4.5; Andersen, *et al.*, 2010; Prada *et al., in review*). Plots at Fortuna were censused using the sample protocol as the lowland plots (Condit, 1998), although the minimum size of trees census differed: For nine of 12 Fortuna plots, all trees $\geq$ 5 cm DBH were measured in 2008. For three plots (Chorro A, Honda B, Hornito), stems $\geq$ 10 cm were measured. For all individuals included in the 2008 census, multiple stems $\geq$ 1 cm DBH were measured.

Soil sampling and analysis

Soil cores were taken to a depth of 10 cm from 13 locations in each 1-ha plot during the wet season at both lowland and montane sites. Bulk density was determined by drying a known volume of soil at 105°C. Soil pH was determined in a 1:2 soil to deionized water ratio using a glass electrode. Total soil inorganic N was calculated as the sum of soil nitrate and ammonium measured in 0.5 M K_2SO_4 extracts and determined by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Readily exchangeable P, which approximates plant available P, hereafter "soil resin P", was determined by extraction with anion-exchange membranes (Turner & Romero, 2009). Base cations were extracted in Mehlich-3 solution

(Mehlich, 1984) with detection by ICP–OES on an Optima 7300 DV spectrometer (Perkin-Elmer, Shelton, CT).

Assessing multiple stems as a functional trait

To determine if multiple stem frequency is a reliable proxy of resprouting strategies, we used the long term BCI 50 ha plot census data. These data record incidences where the main stem was broken below 1.3 m and replaced by a resprout stem (census code = "R") or where trees were broken above 1.3 m, and survived, presumably, by the creation of resprouts above 1.3 m (census code = "Q"), which occurs frequently in broken trees on BCI (Putz *et al.*, 1983). For undamaged trees present in the first BCI census (1982) and still alive in the most recent census (2010), we categorized trees that transitioned to code "Q" or "R" at any of the six census intervals "resprouts". We fit a generalized linear model with a binomial error distribution to evaluate if tree size (DBH) and stem status (multiple- or single-stemmed) was associated with the probability of resprouting for trees present in the first BCI census. We specified resprout (yes or no) as the dependent variable and the interaction between log(DBH) and stem status as the fixed effect. We present coefficients of this linear model and the analysis of deviance table for the type "II" test of fixed effects.

We evaluated if taxonomy explains significant variance in multiple stem frequency using multi-level linear models specifying presence of multiple stems as the dependent variable and species, genus, and family as nested random effects. For each taxonomic level we calculated the variance partitioning coefficient (VPC; Goldstein *et al.*, 2002), which, for species (VPC_s), would be calculated:

$$VPC_s = \sigma_s /(\sigma_g + \sigma_f + \sigma_e)$$

where $\sigma_{s,}$ σ_g, σ_f are the variance explained by the species, genus, and family level random effects, respectively, and σ_e is the variance of the residual error term. The use of multi-level models to partition variance is typically recommended for data with a normal error distribution, but a linear model can be applied to partition variance in discrete response variables where the probability of the target variable is not close to 0 or 1 (Goldstein *et al.*, 2002). We evaluated this model for the 2005 BCI census in four size classes: 1-5 cm, 5-10 cm, $\geq$ 5 cm, and $\geq$ 10 cm. Mixed-effect models were fit using *lme4* in R (Bates *et al.*, 2014).

Functional trait datasets

To evaluate how species multiple stem frequency co-varies with demographic rates, wood density, and nutrient allocation & acquisition, we assembled two functional trait datasets for tree species at BCI and Fortuna. We list the availability of covariates for each dataset in Table 4.2. Variance partitioning and the resprouting analyses indicated that the presence of multiple stems in saplings 1-5 cm in DBH poorly predicts resprouting rates and is not well constrained at the species level (see Results section). Consequently, species multiple stem frequency was calculated as the proportion of individuals $\geq$ 5 cm DBH with at least one multiple stem $\geq$ 1 cm DBH in each census, and then averaged across available census intervals. RGR was calculated as $(\ln(DBH_{time2})-\ln(DBH_{time1}))/(time2 - time1)$, and mortality rate was calculated as $(\ln(N_{time1})-\ln(N_{time2}))/(time2 - time1)$, where N_{time1} is is the number of live individuals at time 1 and N_{time2} is the number of individuals alive in time 1 that survived to time 2. Multiple stems frequency, and demographic rates at Fortuna were calculated using census data pooled across the 12 1-ha plots. Species RGR was calculated for the Fortuna dataset as the average of species mean growth rates across the two available census intervals 2003-2008 and 2008-2013, and RGR and mortality rates for BCI species were averaged across the six available census intervals.

Mortality rates were not determined for Fortuna species because of the relatively small number of individuals per species available. In the BCI dataset, we included species soil P association coefficients (P_{hab}), which were determined in Condit *et al.*, (2013) from a hierarchical bayesian logistic regression model used to evaluate the environmental correlates of tree species distributions across 1-ha plots and species inventory transects in the Panama Canal watershed. Condit *et al.* (2013) use this coefficient to divide species into three groups: positive P affinity ($P_{hab} \geq 0.5$), negative P affinity ($P_{hab} \leq -0.5$), and neutral P affinity ($-0.5 < P_{hab} < 0.5$). Wood density values for 152 BCI species were available from Wright *et al.,* 2010 accessed through the Global Wood Density Database (Zanne *et al.,* 2009). Foliar N and P concentrations for shade leaves were determined for all tree species on BCI, and have been previously presented in (Dalling *et al*., 2016). Species mean wood density and N and P concentrations of the outer 5 cm of sapwood and shade leaves were determined directly for 56 tree species at Fortuna as described in Heineman *et al*., (2016). At both Fortuna and BCI, three shade leaves were collected from three individuals per species, and the tissue for each individual was ground together prior to chemical analysis. If a functional trait for a species were measured in more than one Fortuna plot, we used the mean value across all plots. In the final Fortuna and BCI datasets, we excluded species with fewer than 50 individuals $\geq$ 5 cm DBH in the 2005 BCI census or 2008 Fortuna census to ensure that life history parameters were well estimated. Our final trait datasets consisting of species with all available covariates included 71 species for BCI and 43 species for Fortuna.

Multivariate analysis of functional traits

For both species trait datasets, we used principal component analysis to determine the multivariate relationships among species traits. Pearson correlation coefficients were calculated

for each pairwise trait relationship. Wood nutrients, foliar nutrients, RGR, and mortality rates were log transformed and multiple stem frequency (which included zero values) was square root transformed prior to principal component and regression analyses. For the BCI dataset, we used an analysis of variance to determine if multiple stem frequency differed among species with negative, positive, and neutral soil P associations.

Evaluating forest-level variation in multiple stem frequency

For each 1-ha plot, multiple stem frequency was assessed as the percentage of individuals with a principal stem > 10 cm DBH and at least one multiple stem > 1 cm DBH. We calculated this metric both including and excluding arboreal palms, as palms do not have secondary meristems, and therefore, do not resprout in the same way as angiosperm or gymnosperm trees. In addition, *Oenocarpus mapora,* a common palm in this region forms clumps of clonal stems (De Steven, 1989), but this habit is physiologically distinct from the axillary resprouting common to tropical tree species (Bellingham & Sparrow, 2000).

To determine if variation in multiple stem frequency among forest plots correlates with environmental parameters, we fitted linear regression models between multiple stem frequency and the first two soil principal component axes derived from soil resin P, Mehlich Ca, K, Mg, soil inorganic N, and soil pH, which together explain 71% of the variance (Table 4.6). We also tested the relationship between multiple stem frequency and mean annual precipitation and mean monthly precipitation during the drier half of the year (December-May) as reported for the Panama Canal sites on (CTFS webpage) and for Fortuna in Prada *et al.* in review. We used an analysis of variance to determine if multiple stem frequency differed among forest stands of different ages in the Panama canal watershed. To determine which specific abiotic variables (soil pH, Ca, K, Mg, NO_3, NH_4, resin P, elevation, MAP, dry season rainfall, and forest age) best

predict plot-level multiple stem frequency among plots, we used Least Absolution Shrinkage and Selection Operator (LASSO) variable selection procedure using the *cv.glmnet* function in the package *glmnet* in R. LASSO is a penalized regression method that can be used for variable selection among correlated predictors because, in contrast to more commonly used regression methods, parameters that have little explanatory power are shrunk to zero. Our final regression model included only the covariates included in the LASSO model with the lowest lambda value. Soil P and cations were log transformed prior to principal component analysis and LASSO to meet distributional assumptions.

RESULTS

Multiple stems as predictor of resprouting

Tree size and stem status (multiple or single stemmed) were both significantly related to the probability that a tree present in the 1982 BCI census would resprout in one or more of six subsequent census intervals. For trees smaller than 5 cm, 30% of trees with multiple stems in 1980 census (that survived until 2010) were recorded as a resprout in a succeeding plot census, compared to 22% of single-stemmed individuals. For tree greater than 10 cm, this discrepancy increased, as the probability of resprouting multi-stemmed trees (45%) was three times greater than the probability for single stemmed trees (15%). In line with this pattern, we found a significant interaction between stem status and DBH in our generalized linear model (F = 179.96, $P < 0.001$; Table S4). This interaction indicates that while the smallest trees had a similar probability of resprouting regardless of stem status, the probability of resprouting declined sharply with DBH in single stemmed trees, whereas resprouting probability increased slightly with DBH in multiple-stemmed trees (Figure 4.2).

Taxonomic and size class variation in multiple stem frequency

The frequency of multi-stemmed trees, and taxonomy control over multiple stem frequency varied among size classes on Barro Colorado Island (Table 4.1). Trees in the smallest size class (1-5 cm) had the highest proportion of individuals with multiple stems (10.9%), whereas the proportion of multi-stemmed trees in the largest size class examined (>10 cm DBH) was the lowest (5.8%). While the overall frequency of multi-stemmed trees declined with size, the proportion of variance in multiple stem frequency explained by taxonomy was greater for large trees compared to small trees. Across all trees > 1 cm in the 2005 BCI census, species, genus, and family together explained 17% of the variation in multiple stem frequency. For trees 1-5 cm DBH, taxonomic levels explained 11% of variance in multiple stem frequency. The proportion of variance explained by taxonomy increased to 25% when evaluated for tree 5-10 cm, 34% for trees > 5 cm, and 52% for trees > 10 cm. Genus explained the greatest proportion of variance among taxonomic levels for all size classes except for trees > 10 cm, for which species had the highest VPC (0.31; Table 4.1). Together these results suggest that while nearly all species are capable of maintaining multiple stems as juveniles, only some species retain the ability to create multiple stems as pole-sized and larger trees. For the following species level results, we evaluate multiple stem frequency for trees > 5 cm because very little variance was explained by taxonomy in saplings (1-5 cm) and too few trees > 10 cm are present to constrain species estimates.

Species multiple stem frequencies ranged between 0.2 and 60% across 71 species examined in BCI and between 0 and 63% for 43 species examined at Fortuna. Multiple stem frequency was log-normally distributed across species with two-thirds of species across both

datasets having multiple stem frequencies of < 10% and nine species having no individuals with multiple stems.

Multivariate trait relationships

In the principal component analysis of the 71 species in the BCI trait dataset, the first three principal component (PC) axes together explained 66% in variance among species. P_{hab}, foliar N, and foliar P co-varied significantly (Table 4.8) and loaded strongly in the same direction on the first PC axis (PC1), which explained 32% of the variation among species (Figure 4.3a, Table 4.8). The second PC axis (PC2) explained 20% of variance and represented a previously described growth-mortality axis, which positively correlated with wood density and negatively correlated with growth and mortality rates. Contrary to our hypothesis, multiple stem frequency did not load strongly on either axis, but instead was significant positively correlated with the PC3 (Figure 4.3a, Table 4.8), which explained 14% of variance. The multivariate relationships among traits in the Fortuna dataset were similar to those observed in the BCI dataset (Figure 4.3b). Among 43 Fortuna species, tissue nutrient concentrations co-varied in the same direction as multiple stems on the first PC axis which explained 46% of variance among species, and RGR and wood density loaded in opposite directions on the second PC axis which explained 18% of the variance. Multiple stem frequency loaded most strongly on the third axis, which explained 13% of the variance (Table 4.8).

Examination of pairwise trait relationships revealed that species soil P association coefficient, or P_{hab} was the only trait significantly correlated with multiple stem frequency (Table 3; Figure 4a), although this relationship was fairly weak. Foliar N and P were not significantly associated with multiple stem frequency (Figure 4.4b, Table 4.3). When species were grouped by P_{hab} values into positive, negative, and neutral soil P affinity groups, there was no significant

effect of soil association group on species multiple stem frequency in trees >5 cm ($F_{2,68}$ = 1.76, P = 0.18; Figure 4.5a). However, the effect of soil association on the species frequency of multiple stems in trees > 10 cm was significant ($F_{2,46}$ = 4.64, P = 0.014): species with a positive P affinity had a higher proportion of multiple stems (11% ± 4%) than species with neutral (3.8% ± 0.9%) or negative (1.3 % ± 0.4%) soil P affinities (Figure 4.5b). In contrast to BCI, three of four nutrient allocation traits examined (foliar P, wood P, and wood N) were significantly positively correlated with species multiple stem frequency among species across the fertility gradient at Fortuna (Figure 4.4c-d). Similar to the BCI dataset, multiple stem frequency was not significantly related to wood density or RGR at Fortuna (Table 4.4).

Forest-level multiple stem frequency

There was considerable variation among forest plots in the proportion of woody dicot individuals > 10 cm with at least one multiple stem. Multiple stem frequency ranged between 0.9 and 21.3% across 49 Panamanian plots with an average frequency of 9.0% ± 0.7%. There was no significant difference in the average multiple stem frequency between lowland plots (8.9% ± 0.9%) and lower montane plots (9.5% ± 0.9%) plots.

When montane plots (all old growth) and lowland plots were combined in the same model, there was no significant effect of forest age on multiple stem frequency of woody trees ($F_{2,46}$ = 2.78, P= 0.072; Figure 4.6). However, when lowland plots were analyzed alone, there was a significant effect of forest age ($F_{2,34}$ = 5.39, P = 0.009), with old growth plots having lower average multiple stem frequency (4.2% ± 0.5%, N = 9 plots) than mature secondary (11.0% ± 1.5%, N = 14 plots) or young secondary plots (9.8% ± 1.5%, N = 14 plots). There was also a significant effect of forest age on several soil attributes: old growth forest had lower

average soil K and NH_4 concentrations than mature secondary forest, and lower soil resin P concentrations than both mature and young secondary forest (Table 4.9).

Across both lowland and montane forest plots, multiple stem frequency at the level of the tree community increased with soil fertility, as multiple stem frequency was significantly positively correlated with soil PC1 (r^2 = 0.25, df = 1,47, P < 0.001; Fig. 4.6a), which was positively associated with measures of soil fertility including soil pH and soil resin P, NO_3, Ca, K, and Mg concentrations (Table 4.6). Soil PC2, on which soil NH_4 loaded most strongly, was not significantly correlated with multiple stem frequency (r^2 = 0.0, df = 1,47, P = 0.26). Multiple stem frequency was not significantly correlated with mean annual precipitation (r^2=0.0, df = 1,47, P = 0.923) or dry season rainfall (r^2=0.0, df = 1,47, P = 0.864) across all plots, nor were these variables significant when the effect of rainfall on multiple stem frequency was evaluated for seasonally dry lowland plots or wet montane plots separately.

When LASSO variable selection was used to identify which specific environmental variables best predict multiple stem frequency, soil resin P was the only variable included in the model with the lowest lambda value (lambda = 0.578). Multiple stem frequency increased significantly with log transformed soil resin P (r^2 = 0.44, df = 1,47, P < 0.001; Figure 4.6b). When we excluded secondary growth plots, which had significant higher soil resin P than the mature forest plots, the relationship between multiple stem frequency and soil resin P remained significant (r^2 = 0.31, df = 1,19, P < 0.005).

DISCUSSION

Multiple stems as a functional trait

Resprouting is an integral part of tree life history that influences plant fitness (Bond & Midgley, 2003); however, experimental tests of how tree species resprouting tendencies vary

within and among communities are not feasible in high diversity tropical forests. In this study, we demonstrate that the frequency of multiple stems recorded in inventories of permanent forest plots provides a proxy of tree species resprouting strategies in tropical forest communities, although this measure is perhaps more meaningful for large trees compared to small trees. For the smallest trees in the BCI census, the probability that an undamaged tree alive in 1982 census would subsequently resprout and survive to 2010 was approximately equal for single- and multi-stemmed individuals (~30%). However, for trees larger than 10 cm, the probability that a single-stemmed tree would resprout in subsequent census intervals (15%) was three times lower than the probability of resprouting for multi-stemmed trees (46%). These results are consistent with a previous study which tracked the survival of snapped or broken trees on BCI and found that the ability to survive damage significantly declined with increasing diameter (Putz & Brokaw, 1989). Our results also suggest that this ontogenetic shift in resprouting abilities varies among taxa, as the proportion of variance in multiple stem frequency that could be explained by taxonomy increased with size, supporting previous findings that while most trees species can resprout as juveniles, the ability to resprouting as adults is highly species specific (Del Tredici, 2001; Shibata *et al.*, 2014). Consequently, multiple stem frequency may best differentiate resprouting patterns among species for tree larger than saplings. There are undoubtedly methodological limitations to using multiple stem frequency from plot data as a metric of resprouting. For instance, this method would underestimate the frequency of multiple stems in species that form secondary stems above 1.3 m. However, given the logistical difficulties in assessing species and forest resprouting tendencies experimentally, this approach has potential to provide novel insights into broad scale ecological correlates of resprouting in tropical forest communities.

Species correlates of multiple stem frequency

In line with previous global patterns of resprouting in low disturbance plant communities multiple stem frequencies in Panamanian tree species varied continuously, rather than falling into dichotomous "sprouter" and "nonsprouter" categories (Vesk & Westoby, 2004). The 60-fold variation in multiple stem frequencies among species at both BCI and Fortuna forest indicated that there are biologically meaningful differences in the vegetative regrowth strategies that influence plant fitness and ecology. Despite both sites displaying high interspecific variation in multiple stem frequency, we found no evidence for the widely held hypothesis that resprouting is a persistence strategy (Bond & Midgley, 2001; Bellingham & Sparrow, 2009), aligned with slow growth, low mortality rates and high wood density among tropical tree species (Poorter, Kitajima*, et al.*, 2010). This finding supports previous studies that resprouting strategies are unrelated to demographic rates in tree species (Dietze & Clark, 2008; Shibata *et al.*, 2014). While it seems counter intuitive that resprouting abilities should be unrelated to species mortality rates, the positive effect of resprouting on survival may be confounded if species that resprout are more likely to experience the forms of damage that induce resprouting. For instance, trees on BCI with slow growth rates and high wood density are less likely to experience stem snapping than trees with low wood density (Putz *et al.*, 1983). Therefore, tree species with high wood density could be more likely to survive a tree fall but less likely to experience a resprout-inducing event than trees with fast-growing trees with low wood density.

While the importance of demography and carbon allocation on resprouting strategies has been extensively examined, the importance of nutrient storage and remobilization in resprouting responses has received little attention, despite widespread evidence that production of leaf and woody biomass in tropical forests is limited by the one or more soil nutrients (Vitousek, 1984;

Tanner *et al.*, 1998; Wright *et al.*, 2011). We found mixed support for our hypothesis that multiple stem frequency should be associated with the concentrations of nutrients limiting foliar and woody biomass growth. Among co-occurring species on BCI, species multiple stem frequency was not correlated with foliar N and P concentrations, whereas multiple stem frequency correlated significantly with foliar P, wood P, and wood N in species sampled across a soil gradient at Fortuna. The strong relationship between wood nutrients and multiple stem frequency at Fortuna supports previous findings that resprouting in woody plant requires remobilization of nutrient reserves from above and below ground woody biomass (El Omari *et al.*, 2003; Kabeya & Sakai, 2005). However, it is difficult to assess whether the relationship is driven by species differences in nutrient allocation or environmental differences nutrient availability. The weak, but significantly, correlated between multiple-stem frequency and species soil P association in Panama, and the significant with correlation with wood P in Fortuna where there is high interspecific variation within a single site, suggests that resprouting is related to species P allocation and acquisition strategies. However, soil nutrient availability explains a significant proportion of interspecific variation in the concentrations of P in foliar (Fyllas *et al.*, 2009) and woody (Heineman *et al.*, 2016) biomass in tropical forests, indicating that P allocation cannot be separated from P availability. Consequently, the result that multiple stem frequency associated with plant nutrient concentrations when species differed in access to nutrients (Fortuna) and not among species co-occurring on the same soil habitat (BCI) suggests that multiple stem frequency may be more closely related to nutrient availability than species evolutionarily prescribed allocation patterns. Furthermore, in multivariate trait analyses, multiple stem frequency loaded on an axis that was orthogonal to those represented by nutrient allocation parameters and demographic rates, suggesting multiple stem variation among species may be

more strongly influence by features not measured here, such as bud position and number (Clarke *et al.*, 2013) or carbohydrate storage (Kabeya & Sakai, 2005).

Community correlates of multiple stem frequency

While the relationship between species nutrient allocation and multiple stem frequency was complex, the community level relationship with soil nutrients was clear: the proportion of woody dicots > 10 cm DBH with more than one stem increased with soil fertility across the 47 forest Panamanian forest plots. This result is in stark contrast to the existing theoretical paradigm, that as a persistence strategy in wood plants, multiple stems should be more common on low resource environments (Bellingham & Sparrow, 2000; Bond & Midgley, 2001). Empirical support for this paradigm is in forests is mixed, as Bellingham & Sparrow (2009) found a negative relationship between soil P and multiple stem frequency in Jamaican forests, but a positive relationship between multiple stem frequency with soil P for plots in New Zealand. Our study is the most robust examination of multiple-stem frequency in tropical forests to date given that the plots evaluated are relatively large (1-ha) and the steep fertility gradient evaluated here encompasses the total range of soil nutrient availabilities observed throughout the tropics (Gartlan *et al.*, 1986; Baillie *et al.*, 1987; Phillips *et al.*, 2003, Quesada *et al.*, 2009).

The positive relationship between soil nutrients and multiple stems is more consistent with studies of high disturbance ecosystems, such as Mediterranean shrublands where soil nitrogen availability has been shown to be more important than nutrient and carbohydrate reserve storage in predicting resprouting responses (Cruz *et al.*, 2003; Knox & Clarke, 2005). Furthermore, across frequently burned landscapes in Australia, soil fertility is a better predictor of resprouting than disturbance regime (Clarke *et al.*, 2005). The relationship between multiple stems and soil fertility may also meet theoretical expectations: models of evolutionarily stable

tree allocation strategies predict that as forests shift from nutrient-limitation to light-limitation along fertility gradients, carbon allocation shifts from roots to woody biomass (Dybzinski *et al.*, 2011). Consequently, the production of multiple stems may occur as a consequence of release from nutrient limitation in these forests where the production of foliar tissues will only exacerbate light limitation. Furthermore, forests growing on high fertility soils typically have high growth and mortality rates (Russo *et al.*, 2008), and, therefore, higher canopy turnover than forests on low fertility soils (Quesada *et al.*, 2009). The creation of multiple stems on high-fertility soils may therefore have the added benefit of mitigating damage from tree-falls.

Because the concentrations of soil nutrients strongly co-vary both in soil and in plant tissue due to stoichiometric principles, it is impossible to determine from our data if a specific nutrient is responsible for the observed pattern. However, variable selection methods indicated that soil P was the best predictor of community multiple stem frequency. Phosphorus has shown to be an important driver of forest structure and function in Panama. Soil P is a strong correlate of tree species distributions in both the Canal watershed (Condit *et al.*, 2013) and Fortuna (Prada *et al.,* 2016) and co-limits productivity in a long-term Panamanian nutrient addition experiment (Wright *et al.*, 2011). Phosphorus accumulation in the stems and leaves has been widely observed in nutrient addition experiments in the tropics (Schreeg *et al.*, 2014; Harrington *et al.*, 2001; Ostertag, 2010; Mo *et al.*, 2015), indicating that the quantity of P in mobile reserves should increase with soil P availability. Experimental tests are needed to determine if trees on high fertility soils resprout more due to increase soil nutrient supply directly, or due to increased reserve accumulation where soil nutrient supply is greater.

It should be noted that soil P is significantly lower in primary forest stands than mature and young secondary stands examined in Panama. Resprouting can predominate in the earliest

stages of succession, as resprouted stems are often more abundant than stems that originate from seed in the first few years after forest clearing (Uhl *et al.*, 1981). However, the majority of forest stands included in this study have not experienced human disturbance in over 50 years and are contiguous with large expanses of mature forest (Pyke *et al.*, 2001). Furthermore, the effect of soil is significant when primary and secondary forests were analyzed separately, indicating that the importance of soil nutrients is robust.

Implications for forest dynamics

Community level variation in tree species resprouting strategies have important implications for models evaluating forest dynamics (Loehle, 2000; Dietze & Clark, 2008) because resprouting of existing trees limits the opportunities for new trees to recruit from seeds after disturbance. On BCI, resprouting of canopy trees contributes significantly to the canopy closure after disturbance resulting in gaps smaller than 100 m^2 (Putz & Brokaw, 1989), which make up over 90% of gaps on BCI (Hubbell *et al.*, 1999; Lobo & Dalling, 2014). Variation in multiple stem frequency and resprouting across soil fertility gradients may influence widely studied diversity-productivity relationships (Waide *et al.*, 1999; Mittelbach *et al.*, 2001), given that limited recruitment of seeds in tree fall gaps is considered among the most important forces in maintaining tropical tree species diversity (Hubbell *et al.*, 1999). In addition, resprouting may become an increasingly important force in forest dynamics as disturbance events such as hurricanes (Emanuel, 2005) and forest fires (Cochrane, 2003) are projected to become more frequent and severe in the tropics.

Conclusions

We demonstrate that analyzing the frequency of multiple-stemmed trees in long-term forest plot data provides important insight into the life history of tree species in tropical forests.

While we were unable to identify generalizable species level correlates of multiple stem frequency, the strong relationship between multiple stem frequency and fertility in Panamanian tree communities suggests a novel mechanism by which soil nutrient availability influences the structure and function of tropical forests.

ACKNOWLEDGMENTS

Funding for this research was provided by the National Science Foundation (NSF) graduate research fellowship and NSF doctoral dissertation improvement grant (DEB-1311379). We thank the Center for Tropical Forest Science for providing the forest plot data for the 1-ha plots and BCI 50-ha plot in the Panama Canal watershed, and we thank Suzanne Lao for assisting with data management queries. The BCI forest dynamics research project was founded by S.P. Hubbell and R.B. Foster and is now managed by R. Condit, S. Lao, and R. Perez under the Center for Tropical Forest Science and the Smithsonian Tropical Research in Panama. We thank the Smithsonian Tropical Research Institute Soil Analysis Lab for performing the field sampling and chemical analysis of soil at Panamanian forest sites. We thank Christopher Paciorek for providing correspondence and statistical output from his previous study of resprouting at BCI. We thank Adriana Corrales, Jennifer Jones, and Cecilia Prada for comments that improved this manuscript.

LITERATURE CITED

Andersen KM, Endara MJ, Turner BL, Dalling JW. **2012**. Trait-based community assembly of understory palms along a soil nutrient gradient in a lower montane tropical forest. *Oecologia* **168**, 519-531.

Andersen KM, Turner BL, Dalling JW. **2010**. Soil-based habitat partitioning in understorey palms in lower montane tropical forests. *Journal of Biogeography* **37**, 278-292.

Baillie IC, Ashton PS, Anderson JAR, Fitzpatrick EA, Tinsley J, others. **1987**. Site characteristics and the distribution of tree species in mixed dipterocarp forest on tertiary sediments in central sarawak, malaysia. *Journal of Tropical Ecology* **3**, 201-220.

Bellingham PJ, Sparrow AD. **2000**. Resprouting as a life history strategy in woody plant communities. *Oikos*, 409-416.

Bellingham PJ, Sparrow AD. **2009**. Multi-stemmed trees in montane rain forests: Their frequency and demography in relation to elevation, soil nutrients and disturbance. *Journal of Ecology* **97**, 472-483.

Bond WJ, Midgley JJ. **2001**. Ecology of sprouting in woody plants: The persistence niche. *Trends in ecology & evolution* **16**, 45-51.

Bond WJ, Midgley JJ. **2003**. The evolutionary ecology of sprouting in woody plants. *International Journal of Plant Sciences* **164**, S103-S114.

Cavelier J, Jaramillo M, Solis D, de León D. **1997**. Water balance and nutrient inputs in bulk precipitation in tropical montane cloud forest in panama. *Journal of Hydrology* **193**, 83-96.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. **2009**. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**, 351-366.

Clarke PJ, Knox KJ, Wills KE, Campbell M. **2005**. Landscape patterns of woody plant response to crown fire: Disturbance and productivity influence sprouting ability. *Journal of Ecology* **93**, 544-555.

Clarke PJ, Lawes M, Midgley J, Lamont B, Ojeda F, Burrows G, Enright N, Knox K. **2013**. Resprouting as a key functional trait: How buds, protection and resources drive persistence after fire. *New Phytologist* **197**, 19-35.

Clarke PJ, Lawes MJ, Midgley JJ. **2010**. Resprouting as a key functional trait in woody plants–challenges to developing new organizing principles. *New Phytologist* **188**, 651-654.

Cochrane MA. **2003**. Fire science for rainforests. *Nature* **421**, 913-919.

Condit R. 1998. *Tropical forest census plots: Methods and results from barro colorado island, panama and a comparison with other plots*: Springer Science & Business Media.

Condit R, Engelbrecht BMJ, Pino D, Pérez R, Turner BL. **2013**. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences* **110**, 5064-5068.

Cruz A, Pérez B, Moreno JM. **2003**. Plant stored reserves do not drive resprouting of the lignotuberous shrub erica australis. *New Phytologist* **157**, 251-261.

De Steven D. **1989**. Genet and ramet demography of oenocarpus mapora ssp. Mapora, a clonal palm of panamanian tropical moist forest. *The Journal of Ecology*, 579-596.

Del Tredici P. **2001**. Sprouting in temperate trees: A morphological and ecological review. *The Botanical Review* **67**, 121-140.

Dietze MC, Clark JS. **2008**. Changing the gap dynamics paradigm: Vegetative regeneration control on forest response to disturbance. *Ecological Monographs* **78**, 331-347.

Dybzinski R, Farrior C, Wolf A, Reich PB, Pacala SW. **2011**. Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for light and nitrogen: An analytically tractable, individual-based model and quantitative comparisons to data. *The American Naturalist* **177**, 153-166.

El Omari B, Aranda X, Verdaguer D, Pascual G, Fleck I. **2003**. Resource remobilization in quercus ilex l. Resprouts. *Plant and Soil* **252**, 349-357.

Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. **2007**. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**, 1135-1142.

Emanuel K. **2005**. Increasing destructiveness of tropical cyclones over the past 30 years. *Nature* **436**, 686-688.

Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP. **2007**. Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* **447**, 80-82.

Engelbrecht BMJ, Dalling JW, Pearson TRH, Wolf RL, Galvez DA, Koehler T, Tyree MT, Kursar TA. **2006**. Short dry spells in the wet season increase mortality of tropical pioneer seedlings. *Oecologia* **148**, 258-269.

Fyllas NM, Patino S, Baker TR, Bielefeld Nardoto G, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna V, Mercado LM et al. 2009. Basin-wide variations in foliar properties of amazonian forest: Phylogeny, soils and climate. *Biogeosciences* **6**, 2677-2708.

Gartlan JS, Newbery DM, Thomas DW, Waterman PG. **1986**. The influence of topography and soil phosphorus on the vegetation of korup forest reserve, cameroun. *Vegetatio* **65**, 131-148.

Goldstein H, Browne W, Rasbash J. **2002**. Partitioning variation in multilevel models. *Understanding Statistics: Statistical Issues in Psychology, Education, and the Social Sciences* **1**, 223-231.

Guariguata MR. **1998**. Response of forest tree saplings to experimental mechanical damage in lowland panama. *Forest Ecology and Management* **102**, 103-111.

Harrington RA, Fownes JH, Vitousek PM. **2001**. Production and resource use efficiencies in n- and p-limited tropical forests: A comparison of responses to long-term fertilization. *Ecosystems* **4**, 646-657.

Hayes P, Turner BL, Lambers H, Laliberté E. **2014**. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology* **102**, 396-410.

Heineman KD, Caballero P, Morris A, Velasquez C, Serrano K, Ramos N, Gonzalez J, Mayorga L, Corre MD, Dalling JW. **2015**. Variation in canopy litterfall along a precipitation and soil fertility gradient in a panamanian lower montane forest. *Biotropica* **47**, 300-309.

Heineman KD, Turner BL, Dalling JW. **2016**. Variation in wood nutrients along a tropical soil fertility gradient. *New Phytologist*.

Hubbell SP, Foster RB, O'Brien ST, Harms K, Condit R, Wechsler B, Wright SJ, De Lao SL. **1999**. Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. *Science* **283**, 554-557.

Kabeya D, Sakai S. **2005**. The relative importance of carbohydrate and nitrogen for the resprouting ability of quercus crispula seedlings. *Annals of Botany* **96**, 479-488.

Katabuchi M, Kurokawa H, Davies SJ, Tan S, Nakashizuka T. **2012**. Soil resource availability shapes community trait structure in a species-rich dipterocarp forest. *Journal of Ecology* **100**, 643-651.

Keeley JE. **1977**. Seed production, seed populations in soil, and seedling production after fire for two congeneric pairs of sprouting and nonsprouting chaparal shrubs. *Ecology*, 820-829.

Kitajima K. **1994**. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* **98**, 419-428.

Kitayama K, Aiba S-I, Takyu M, Majalap N, Wagai R. **2004**. Soil phosphorus fractionation and phosphorus-use efficiency of a bornean tropical montane rain forest during soil aging with podozolization. *Ecosystems* **7**, 259-274.

Knox KJE, Clarke PJ. **2005**. Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seeding shrubs. *Functional Ecology* **19**, 690-698.

Kobe RK. **1997**. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos*, 226-233.

Lasso E, Engelbrecht BM, Dalling JW. **2009**. When sex is not enough: Ecological correlates of resprouting capacity in congeneric tropical forest shrubs. *Oecologia* **161**, 43-56.

Liming FG, Johnston JP. **1944**. Reproduction in oak-hickory forest stands of the missouri ozarks. *Journal of Forestry* **42**, 175-180.

Lobo E, Dalling JW. **2014**. Spatial scale and sampling resolution affect measures of gap disturbance in a lowland tropical forest: Implications for understanding forest regeneration and carbon storage. *Proceedings of the Royal Society of London B: Biological Sciences* **281**, 20133218.

Loehle C. **2000**. Strategy space and the disturbance spectrum: A life-history model for tree species coexistence. *The American Naturalist* **156**, 14-33.

Mehlich A. **1984**. Mehlich 3 soil test extractant: A modification of mehlich 2 extractant. *Communications in Soil Science & Plant Analysis* **15**, 1409-1416.

Merz RW, Boyce SG. **1956**. Age of oak seedlings. *J. For* **54**, 664-775.

Mittelbach GG, Steiner CF, Scheiner SM, Gross KL, Reynolds HL, Waide RB, Willig MR, Dodson SI, Gough L. **2001**. What is the observed relationship between species richness and productivity? *Ecology* **82**, 2381-2396.

Mo Q, Zou B, Li Y, Chen Y, Zhang W, Mao R, Ding Y, Wang J, Lu X, Li X. **2015**. Response of plant nutrient stoichiometry to fertilization varied with plant tissues in a tropical forest. *Scientific reports* **5**, 14605.

Ostertag R. **2010**. Foliar nitrogen and phosphorus accumulation responses after fertilization: An example from nutrient-limited hawaiian forests. *Plant and Soil* **334**, 85-98.

Paciorek CJ, Condit R, Hubbell SP, Foster RB. **2000**. The demographics of resprouting in tree and shrub species of a moist tropical forest. *Journal of Ecology* **88**, 765-777.

Phillips OL, Vargas PN, Monteagudo AL, Cruz AP, Zans M-EC, Sánchez WG, Yli-Halla M, Rose S. **2003**. Habitat association among amazonian tree species: A landscape-scale approach. *Journal of Ecology* **91**, 757-775.

Poorter L, Kitajima K. **2007**. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology* **88**, 1000-1011.

Poorter L, Kitajima K, Mercado P, Chubiña J, Melgar I, Prins HH. **2010**. Resprouting as a persistence strategy of tropical forest trees: Relations with carbohydrate storage and shade tolerance. *Ecology* **91**, 2613-2627.

Putz FE, Brokaw NVL. **1989**. Sprouting of broken trees on barro colorado island, panama. *Ecology*, 508-512.

Putz FE, Coley PD, Lu K, Montalvo A, Aiello A. **1983**. Uprooting and snapping of trees: Structural determinants and ecological consequences. *Canadian Journal of Forest Research* **13**, 1011-1020.

Pyke CR, Condit R, Aguilar S, Lao S. **2001**. Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science* **12**, 553-566.

Quesada CA, Lloyd J, Schwarz M, Baker TR, Phillips OL, Patiño S, Czimczik C, Hodnett MG, Herrera R, Arneth A et al. 2009. Regional and large-scale patterns in amazon forest structure and function are mediated by variations in soil physical and chemical properties. *Biogeosciences Discussions* **6**, 3993-4057.

Russo SE, Brown P, Tan S, Davies SJ. **2008**. Interspecific demographic trade-offs and soil-related habitat associations of tree species along resource gradients. *Journal of Ecology* **96**, 192-203.

Sakai A, Sakai S. **1998**. A test for the resource remobilization hypothesis: Tree sprouting using carbohydrates from above-ground parts. *Annals of Botany* **82**, 213-216.

Schreeg LA, Santiago LS, Wright SJ, Turner BL. **2014**. Stem, root, and older leaf n:P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology* **95**, 2062-2068.

Shenkin A, Bolker B, Peña-Claros M, Licona JC, Putz FE. **2015**. Fates of trees damaged by logging in amazonian bolivia. *Forest Ecology and Management* **357**, 50-59.

Shibata R, Shibata M, Tanaka H, Iida S, Masaki T, Hatta F, Kurokawa H, Nakashizuka T. **2014**. Interspecific variation in the size-dependent resprouting ability of temperate woody species and its adaptive significance. *Journal of Ecology* **102**, 209-220.

Tanner EVJ. **1977**. Four montane rain forests of jamaica: A quantitative characterization of the floristics, the soils and the foliar mineral levels, and a discussion of the interrelations. *The Journal of Ecology* **65**, 883-918.

Tanner EVJ, Vitousek PM, Cuevas E. **1998**. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**, 10-22.

Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. **2007**. Controls over foliar n:P ratios in tropical rain forests. *Ecology* **88**, 107-118.

Turner BL. **2010**. Variation in ph optima of hydrolytic enzyme activities in tropical rain forest soils. *Applied and environmental microbiology* **76**, 6485-6493.

Turner BL, Engelbrecht BM. **2011**. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**, 297-315.

Turner BL, Romero TE. **2009**. Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal* **73**, 1972.

Uhl C, Clark K, Clark H, Murphy P. **1981**. Early plant succession after cutting and burning in the upper rio negro region of the amazon basin. *The Journal of Ecology*, 631-649.

Vesk PA, Westoby M. **2004**. Sprouting ability across diverse disturbances and vegetation types worldwide. *Journal of Ecology* **92**, 310-320.

Vitousek PM. **1984**. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **65**, 285-298.

Vitousek PM, Sanford RL. **1986**. Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics*, 137-167.

Waide R, Willig M, Steiner C, Mittelbach G, Gough L, Dodson S, Juday G, Parmenter R. **1999**. The relationship between productivity and species richness. *Annual Review of Ecology and Systematics*, 257-300.

Walker T, Syers JK. **1976**. The fate of phosphorus during pedogenesis. *Geoderma* **15**, 1-19.

Walters MB, Reich PB. **1996**. Are shade tolerance, survival, and growth linked? Low light and nitrogen effects on hardwood seedlings. *Ecology* **77**, 841-853.

Ward W. **1966**. Oak-hardwood reproduction in central pennsylvania. *Journal of Forestry* **64**, 744-749.

Wright SJ, Kitajima K, Kraft NJB, Reich PB, Wright IJ, Bunker DE, Condit R, Dalling JW, Davies SJ, Díaz S et al. 2010. Functional traits and the growth-mortality trade-off in tropical trees. *Ecology* **91**, 3664-3674.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE et al. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616-1625.

Zanne A, Lopez-Gonzalez G, Coomes D, Ilic J, Jansen S, Lewis S, Miller R, Swenson N, Wiemann M, Chave J. **2009**. Global wood density database.

Table 4.1 Variance partitioning coefficients (VPC) from multi-level mixed effect models testing proportion of variance in multiple stem frequency explained by family, genus, and species in size classes of trees in the 2005 Barro Colorado Island census data. We list the number of trees in each size class and the percent of trees in each size class with > 1 multiple stem. Tree counts exclude palms.

	Diameter Size Classes				
	> 1 cm	1-5 cm	5-10 cm	> 5 cm	> 10 cm
Variance Components					
Species	0.07	0.04	0.09	0.13	0.31
Genus	0.08	0.05	0.14	0.16	0.10
Family	0.02	0.01	0.02	0.06	0.11
Species + Genus + Family	0.17	0.11	0.25	0.34	0.52
Residual error	0.83	0.89	0.75	0.66	0.48
Size Class Statistics					
No. of Trees	197283	150452	27372	46831	19459
% Multi-stemmed	10.1	10.9	9.1	7.8	5.8

Table 4.2 Summary of permanent plot census information and availability of species functional trait covariates for two plot networks: Fortuna Forest Reserve in montane western Panama and Barro Colorado Island in the lowland semi-deciduous forest of the Panama Canal watershed.

	Fortuna Plot Network		BCI 50-ha plot	
Census Data				
	Measurements	**Date**	**Measurements**	**Date**
Plot Configuration	12 1-ha plots	Established 2003	One 50-ha plot	Established 1982
Principal Stems	≥ 5 cm (9 plots) ≥ 10 (3 plots)	Fortuna Census 2008	≥ 1 cm	BCI Census 2005
Multiple Stems	≥ 1 cm	Fortuna Census 2008	≥ 1 cm	BCI Census 2005
RGR	Principal stems ≥ 5 cm	Average of 2 census intervals (2003-2013)	Principal stems ≥ 5 cm	Average of 6 census intervals (1982-2010)
Mortality Rates	Not included	Too few individuals & Census intervals	Principal stems ≥ 5 cm	Averaged of 6 census intervals (1982-2010)
Species with ≥ 50 Trees ≥ 5 cm	60	Fortuna Census 2008	114	BCI Census 2005
Species Trait Data				
	Species (N)	**Source**	**Species (N)**	**Source**
Wood Density	79	Heineman et al. 2016	75	Global Wood Density Database
Foliar N & P	109	Heineman et al. 2016	106	Wright et al. 2010
Wood N & P	76	Heineman et al. 2016	Not available	
Soil Habitat Association (P_{hab})	Not available		113	Condit et al. 2013
Total Species with All Covariates	**43**		**71**	

Table 4.3 Correlation matrix describing relationships among functional traits for 71 tree species sampled at Canal watershed. Correlation coefficients in bold are significant at critical $\alpha = 0.05$, and values with an asterisk (*) are significant after the critical threshold has been adjusted for multiple comparisons ($\alpha = 0.004$). Abbreviations: RGR = Relative Growth Rate; Mortality = Mortality rate; Multiples = Multiple Stem Frequency; WD = wood density; P_{hab} = Soil P association (Condit et al. 2013). Tissue nutrient concentrations and demographic rates were log transformed. Multiples was square root transformed

	Leaf P	Leaf N	P_{hab}	WD	RGR	Mortality
Multiples	0.12	0.14	**0.24**	0.00	-0.09	-0.03
Leaf P		**0.72***	**0.53***	**-0.23**	-0.22	-0.08
Leaf N			0.27	0.02	-0.11	0.04
P_{hab}				-0.02	-0.16	-0.17
WD					**-0.26**	-0.05
RGR						**0.27**

Table 4.4 Correlation matrix describing relationships among functional traits for 43 tree species sampled in the Fortuna forest plot network. Correlation coefficients in bold are significant at critical $\alpha = 0.05$, and values with an asterisk (*) are significant after the critical threshold has been adjusted for multiple comparisons ($\alpha = 0.0017$). Abbreviations: RGR = Relative Growth Rate; % Multiples = Multiple Stem Frequency; WD = wood density. Tissue nutrient concentrations and demographic rates were log transformed. Multiples was square root transformed.

	RGR	WD	Leaf P	Wood P	Leaf N	Wood N
Multiples	0.036	-0.047	**0.362**	**0.481***	0.080	**0.437**
RGR		**-0.372**	0.280	0.240	0.272	0.079
WD			-0.263	-0.248	-0.231	-0.162
Leaf P				**0.623***	**0.680***	**0.570***
Wood P					**0.448**	**0.638***
Leaf N						**0.683***

Table 4.5 Site locations and environmental covariates of 1-ha forest plots used in analysis of plot level correlates of multiple stem frequency across 49 permanent forest plots in Panama.

Plot	Network	Age	Latitude	Longitude	Elevation (m)	MAP (mm yr^{-1})	Dry Season Rain (mm mo^{-1})	Soil PC1	Soil PC2
AltoFrio	Fortuna	OG	8.654	-82.215	1100	4641	228	-1.5	1.8
Bonita	Fortuna	OG	8.768	-82.216	1296	5507	415	0.8	0.2
ChorroA	Fortuna	OG	8.749	-82.229	1100	5507	415	2.2	0.1
ChorroB	Fortuna	OG	8.750	-82.233	1239	5507	415	1.5	1.5
HondaA	Fortuna	OG	8.751	-82.239	1155	6255	430	3.1	0.4
HondaB	Fortuna	OG	8.757	-82.244	1241	6159	403	2.2	-0.3
Hornito	Fortuna	OG	8.674	-82.214	1330	5164	260	-1.8	0.8
PaloSeco	Fortuna	OG	8.779	-82.198	878	6257	510	2.3	-0.6
Pinola	Fortuna	OG	8.754	-82.259	1135	4964	296	-1.4	4.3
Samudio	Fortuna	OG	8.731	-82.248	1215	4833	256	1.5	-0.5
VerrugosaA	Fortuna	OG	8.778	-82.180	969	6257	510	2.2	-0.1
VerrugosaB	Fortuna	OG	8.778	-82.170	852	6257	510	2.5	-0.6
Campo Chagres	PCW	YS	9.211	-79.600	109	2481	159	-3.5	-2.0
Caritas	PCW	YS	9.085	-79.608	155	2038	122	-2.5	-0.1
Cerro Galera	PCW	YS	8.927	-79.623	64	1810	103	-2.4	-0.5
Coba1	PCW	OG	9.203	-79.362	515	3104	223	1.0	0.1
Coba2	PCW	OG	9.210	-79.371	643	3084	224	1.0	0.9
Gigante1	PCW	YS	9.099	-79.854	55	2332	150	0.5	0.0
Gigante2	PCW	YS	9.104	-79.854	60	2352	152	-0.1	-0.4
LP1	PCW	MS	9.125	-79.905	80	2535	168	0.5	0.8
LP2	PCW	MS	9.125	-79.885	40	2520	167	-2.2	0.8
P01	PCW	MS	9.333	-79.954	20	2864	196	-1.1	-1.3
P02	PCW	OG	9.323	-79.962	100	2863	196	1.2	1.5

Table 4.5 (cont'd)

P03	PCW	YS	9.260	-79.956	180	2832	195	-1.0	-0.1
P04	PCW	YS	9.258	-79.953	180	2830	195	-1.7	0.8
P05	PCW	MS	9.157	-79.752	40	2325	148	-0.3	-0.7
P06	PCW	MS	9.157	-79.744	30	2311	147	2.2	-1.2
P07	PCW	YS	9.161	-79.743	60	2323	148	2.3	-1.5
P08	PCW	OG	9.168	-79.746	180	2352	151	1.2	-0.1
P09	PCW	OG	9.169	-79.741	160	2345	150	0.8	3.3
P10	PCW	MS	9.145	-79.859	90	2564	171	-1.9	-0.4
P11	PCW	MS	9.145	-79.878	60	2602	174	-1.2	-0.1
P12	PCW	MS	9.179	-79.830	10	2595	174	0.9	-0.3
P13	PCW	MS	9.188	-79.821	55	2591	173	-3.1	-0.5
P14	PCW	OG	9.158	-79.861	60	2616	175	-0.4	-0.1
P15	PCW	OG	9.162	-79.745	70	2330	148	-0.3	-0.7
P16	PCW	OG	9.147	-79.713	160	2248	140	0.0	-0.3
P17	PCW	OG	9.152	-79.716	120	2267	142	-0.7	-1.1
P18	PCW	MS	9.143	-79.883	58	2603	174	-1.3	-0.1
P19	PCW	YS	9.199	-79.774	160	2491	164	2.0	-0.3
P20	PCW	YS	9.190	-79.764	160	2446	160	1.7	-0.1
P21	PCW	YS	9.141	-79.694	110	2226	138	-1.7	-0.1
P22	PCW	YS	9.148	-79.693	180	2250	140	-1.9	-0.7
P23	PCW	MS	9.122	-79.673	30	2153	131	-2.8	-0.8
P24	PCW	MS	9.124	-79.677	50	2160	132	-3.1	-0.8
P25	PCW	MS	9.079	-79.799	110	2177	135	3.8	-0.5
P26	PCW	MS	9.076	-79.787	50	2153	133	3.9	-0.7
P27	PCW	YS	9.080	-79.645	180	2037	122	-1.8	0.1
P28	PCW	YS	9.087	-79.642	160	2048	122	-0.7	0.4

Table 4.6 Principal component axes loadings for soil nutrient concentrations (in mg/kg) in the top 10 cm of soil in 49 Panamanian forest plots. Phosphorus and cation concentrations were log transformed to meet assumptions of normality.

	PC1	PC2
NO_3	0.34	0.28
NH_4	0.05	-0.85
log(Resin P)	0.39	0.26
log(Mehlich Ca)	0.47	-0.18
log(Mehlich K)	0.31	-0.13
log(Mehlich Mg)	0.45	-0.24
soil pH	0.46	0.17

Table 4.7 Analysis of deviance table and linear model coefficients for the generalized linear model (GLM) used to test the effects of DBH and Stem Status (single or multi-stemmed) on the probability that trees present in the first census of trees on Barro Colorado Island resprouted during one of more of the subsequent census intervals. The GLM was fit using a binomial error distribution and logit link function.

Analysis of Deviance (Type II F Test)

Factor	SS	Df	F	P
log(DBH)	1569	1	1554	< 0.001
Stem Status	423	1	419	< 0.001
log(DBH) x Stem Status	182	1	180	< 0.001
Residuals	106687	105651		

Generalized Linear Model Coefficients

Stem Status	Intercept	SE	Slope	SE
Single Stem	-0.100	0.031	-0.389	0.010
Multiple Stem	-1.306	0.136	0.172	0.041

Table 4.8 Loadings of functional trait values on the first three principal component axes and the variance in species traits explained by each axis for the BCI trait dataset (N = 71 species) and the Fortuna trait dataset (N = 43 species). Abbreviations: Multiples = square root(Multiple stem frequency), RGR = log(Relative Growth Rate); Mortality = log(Mortality Rate). Foliar and wood nutrient concentrations were log transformed prior to principal component analysis.

	PC1	**PC2**	**PC3**
BCI Trait Loadings			
Multiples	-0.23	-0.06	0.78
Foliar P	-0.60	0.22	-0.19
Foliar N	-0.50	0.21	-0.38
P_{hab}	-0.48	-0.04	0.23
RGR	0.26	0.58	0.16
Mortality	0.19	0.50	-0.19
Wood Density	0.07	-0.57	-0.31
Variance Explained	**0.32**	**0.20**	**0.14**
Fortuna Trait Loadings			
Multiples	-0.28	-0.44	0.65
Foliar P	-0.47	0.00	-0.14
Foliar N	-0.43	0.10	-0.57
Wood P	-0.45	-0.14	0.21
Wood N	-0.46	-0.26	-0.20
RGR	-0.22	0.62	0.24
Wood Density	0.22	-0.57	-0.31
Variance Explained	**0.46**	**0.18**	**0.13**

Table 4.9 Mean environmental variables of Panama Canal watershed 1-ha plots plots by forest age. Letters correspond to significant differences among forest types as decided by Tukey Honest Significant Difference Test. DBH_{max} refers the DBH of the tree in the 95^{th} quantile for each forest plot.

Age	Young Secondary (N=14 plots)	Mature Secondary (N= 14 plots)	Old Growth (N=9 plots)
DBH_{max} (mm)	571	521	531
Dry Season Precip (mm/mo)	148	159	172
Mean Annual Precip (mm/yr)	2321	2439	2578
Soil Variables			
pH	5.86	5.77	5.55
Resin P (mg/kg)	**4.39[a]**	**4.90[a]**	**1.11[b]**
Mehlich Ca (mg/kg)	2841	2379	1562
Mehlich K (mg/kg)	**88[ab]**	**132[a]**	**42[b]**
Mehlich Mg (mg/kg)	577	585	398
NO3	2.43	2.14	2.20
NH4	**1.37[ab]**	**1.19[a]**	**2.02[b]**
Soil PC1	-0.78	-0.40	0.43
Soil PC2	-0.31	-0.39	0.39

Figure 4.1 Locations of forest sites in including 1-ha plots in the Panama Canal watershed and Fortuna and the 50-ha forest plot on Barro Colorado Island (BCI). Size of plots not to scale.

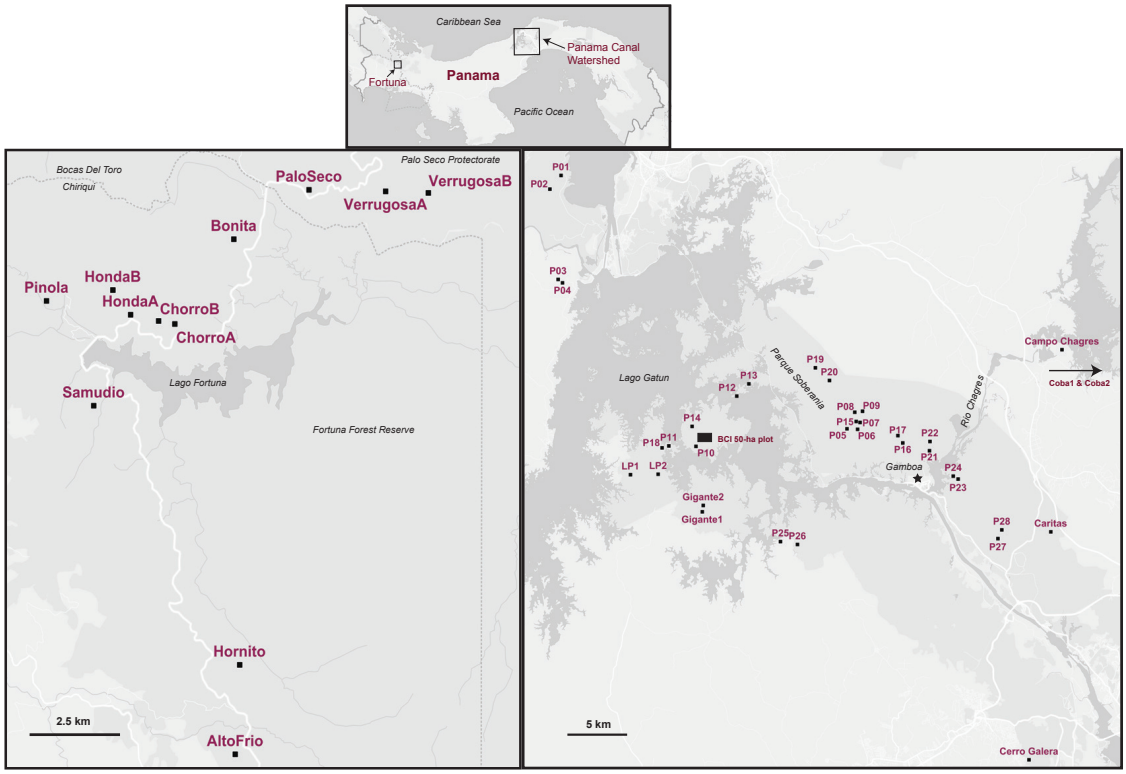


Figure 4.2 Predicted probability that trees present in the first BCI census (1982) resprouted during the course of six subsequent census intervals vs DBH in the 1982 census fit using a generalized linear model testing a log(DBH) x Stem Status interaction effect on resprouting. Trees with multiple stems in the 1982 census are coded in dark blue and trees with one stem in 1982 are coded light blue. Shaded regions around each line represent one standard error. We excluded trees that died during the study interval (1982-2010).

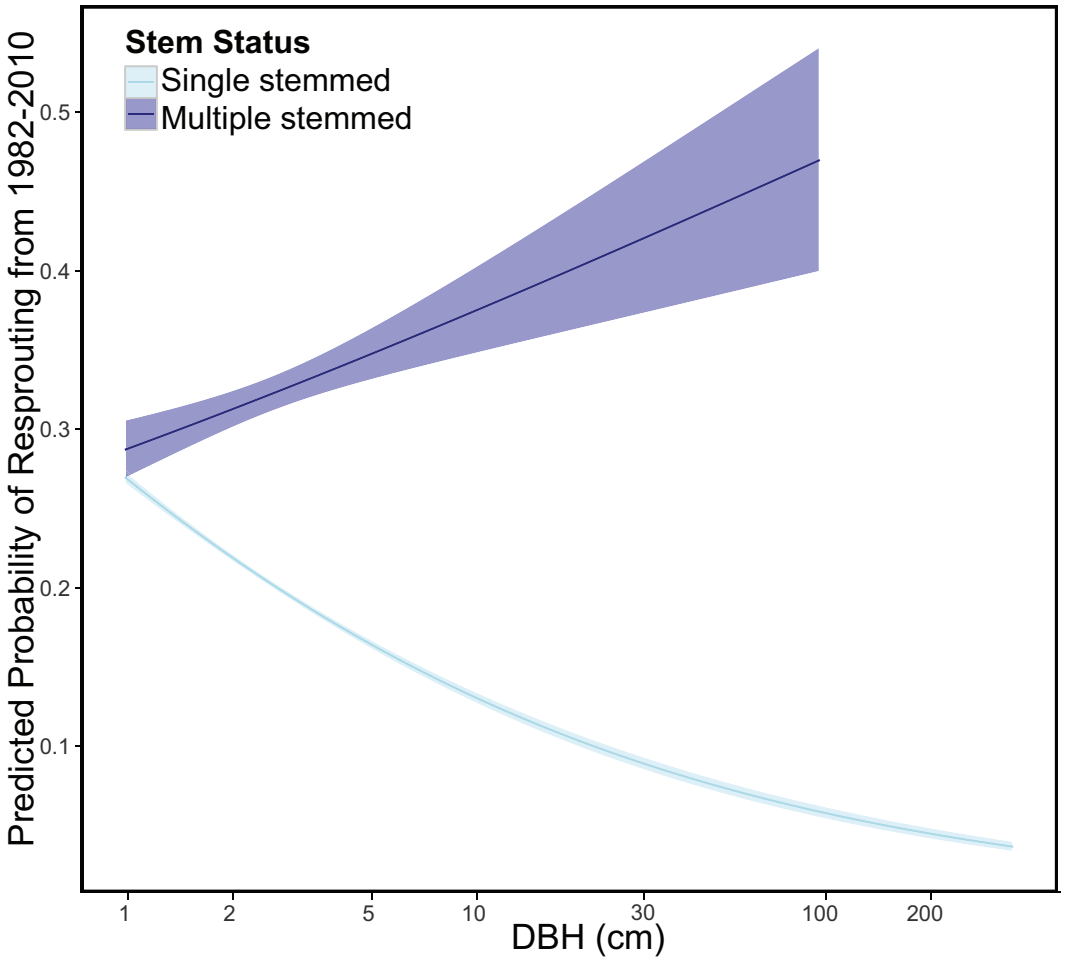


Figure 4.3 Plot of first three principal component axes from multivariate analysis of functional traits for 71 tree species at Barro Colorado Island in the Panama Canal watershed and 43 trees species at Fortuna in western Panama. Abbreviations: RGR = log of Relative Growth Rate; % Multiples = square root(Multiple stem frequency), WD = wood density. Foliar and wood nutrients were log transformed prior to principal component analysis.

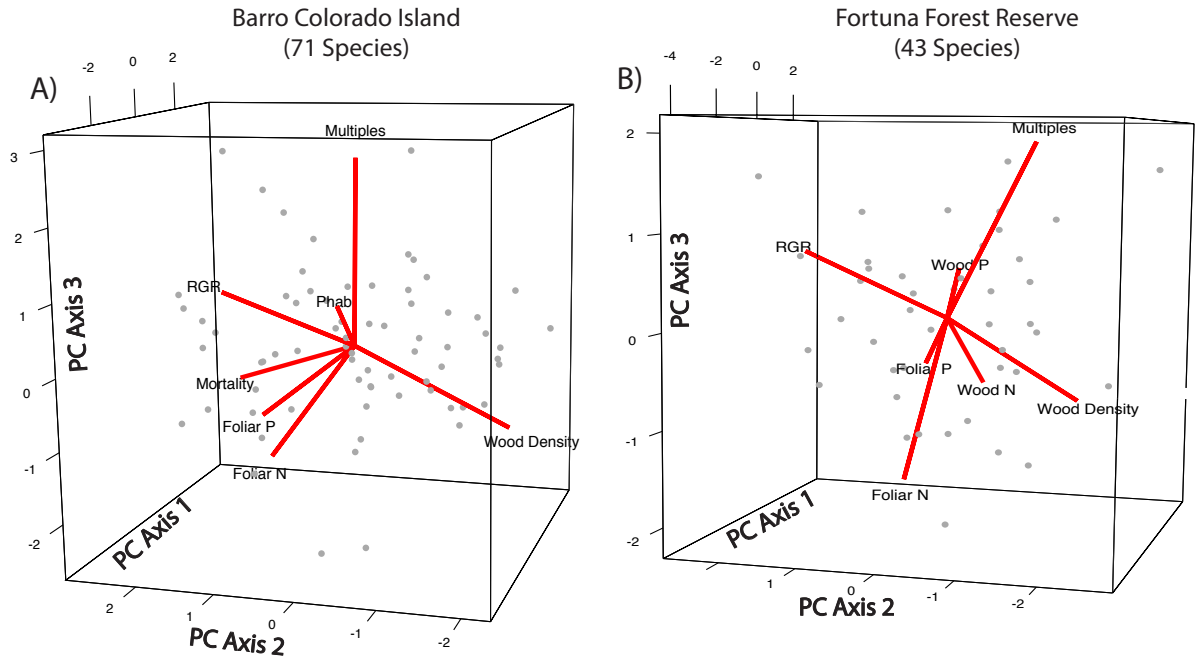


Figure 4.4 The relationship between species multiple stem frequency and phosphorus allocation and association traits for tree species present in the BCI 50-ha plot (a,b) and across the Fortuna plot network (c,d). Species at Fortuna are coded by low, intermediate, and high fertility based on the soil type (Rhyolite (low); Andesite (intermediate); and Dacite (high)) where their relative abundance was the highest.

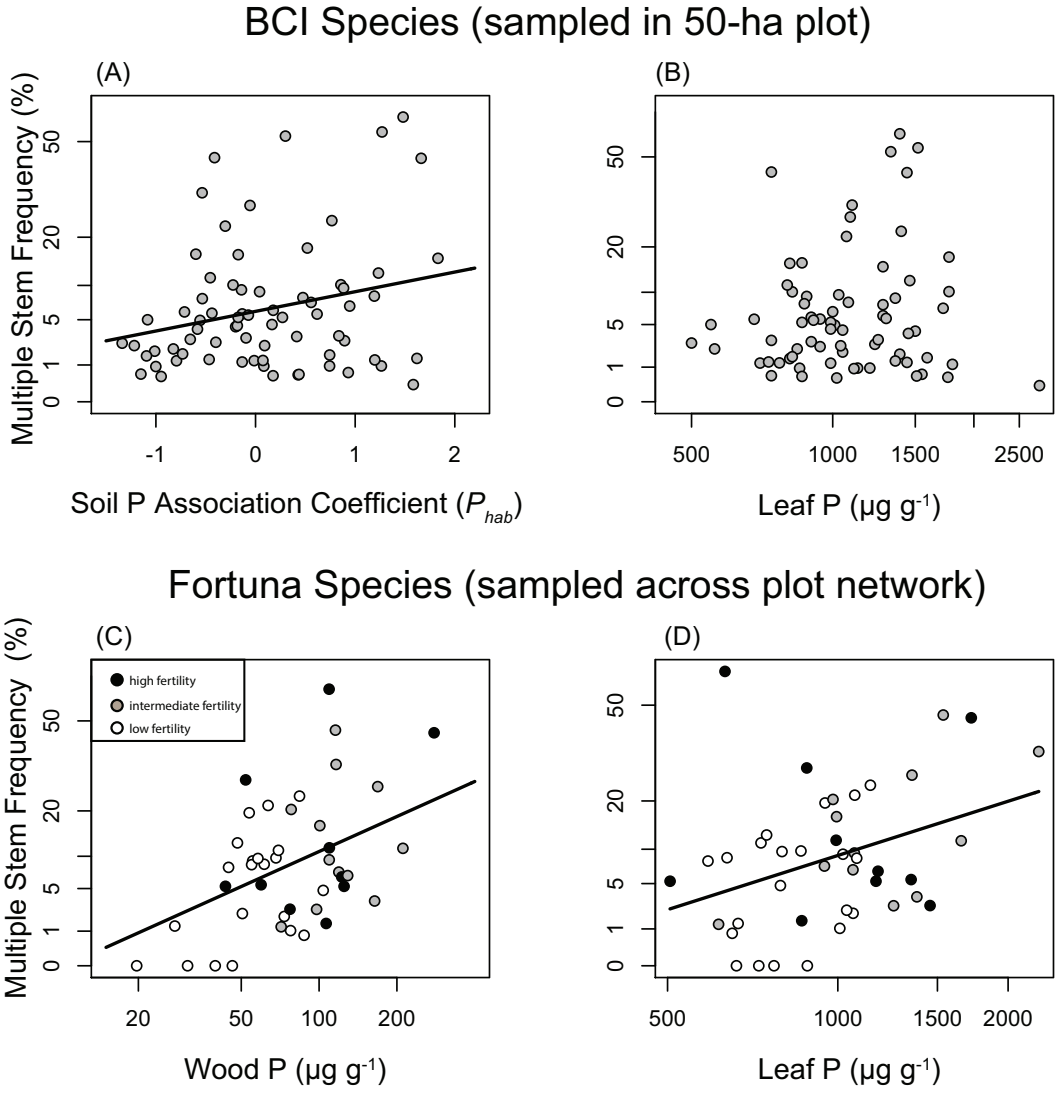


Figure 4.5 Boxplots of species multiple stem frequency grouped by soil phosphorus affinity groups in Condit et al. 2013. Multiple stem frequency was calculated for all individuals per species A) > 5 cm DBH and B) > 10 cm DBH. We eliminated species with fewer than 50 individuals for the given cutoff. N = the number of species included in the affinity group. The y axis is plotted on a square root scale.

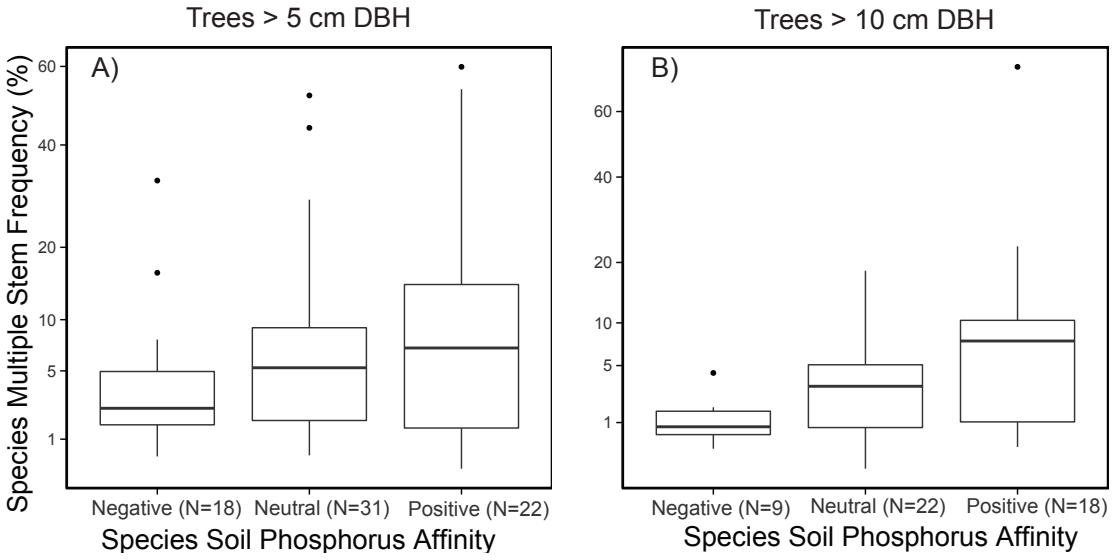
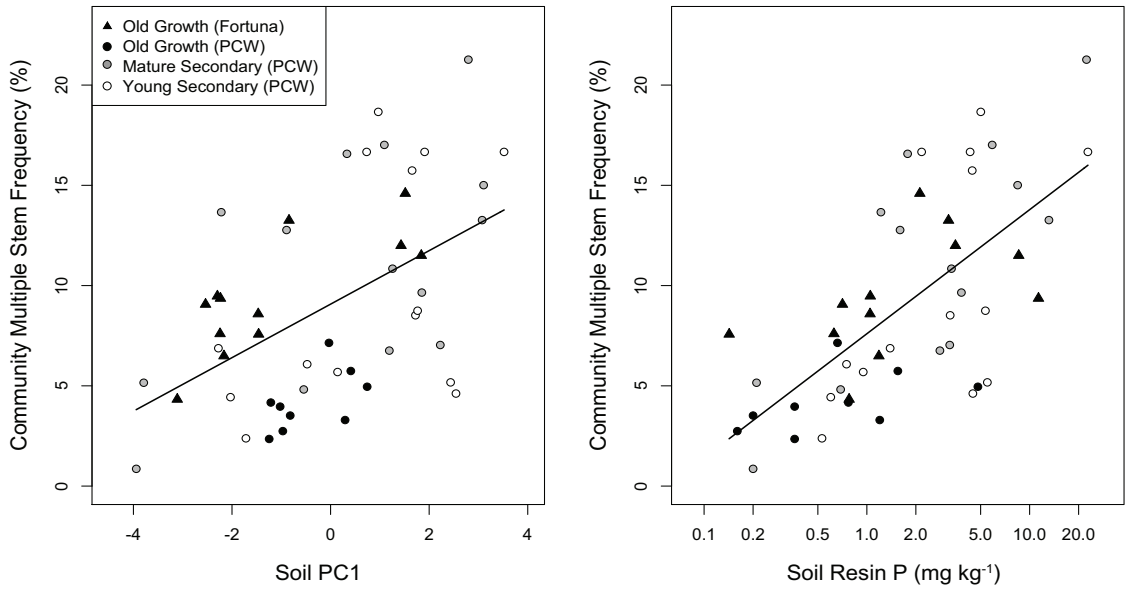


Figure 4.6 The relationship between plot multiple stem frequency (excluding palms) of trees ≥ 10 cm DBH and the first soil principal component axis and with log soil resin P, which was the variable selection as the most predictive of multiple stem frequency by LASSO variable selection. Points are coded by forest age and plot network where Fortuna = 12 montane plots and PCW = 37 Panama Canal watershed plot.



CHAPTER 5

THE IMPORTANCE OF WOOD NITROGEN AND PHOSPHORUS STORAGE IN TROPICAL TREES: EVIDENCE FROM A FIELD-BASED SAPLING DEFOLIATION EXPERIMENT IN PANAMA

ABSTRACT

Access to soil-derived nutrients is critical to tree growth, especially in tropical forests growing on highly weathered, nutrient impoverished soils. However, very little is known about how tropical tree store and remobilize nutrient reserves in stems, despite wood containing the majority of nutrients in tree biomass. Here we evaluate the capacity of tropical trees to remobilize nitrogen and phosphorus from woody biomass by conducting a sapling defoliation experiment including four common tree taxa: *Guarea* (Meliaceae), Legumes (Fabaceae), Nutmeg (Myristiaceae), and *Faramea* (Rubiaceae). We sampled saplings from four habitats including high and low fertility sites in montane and lowland Panamanian forests. To determine the effect of fertility on wood and leaf nutrient stocks, we measured the nitrogen (N) and phosphorus (P) concentrations of foliar and woody biomass prior to defoliation. We then defoliated a group of saplings and measured the wood N and P concentrations after leaf refoliation to determine if trees remobilize N and P from reserves in response for demand to create new leaf tissue. We hypothesized that if storage remobilization is most important where nutrient supply from soil is low, then saplings should remobilize a great proportion of N and P on low versus high fertility soils. We found significant effects of fertility on foliar N and foliar P and wood P concentrations within species, with wood P concentrations being particularly sensitive to soil nutrient

availability. In contrast, concentrations of N in wood did not differ within species between high and low fertility soils, suggesting that wood N is more strongly influenced by taxonomy than soil nutrient supply. We found that even though our defoliation treatment did not substantially increase the leaf area produced in most defoliated relative to control taxa, we observed substantial depletion of wood P reserves in response to leaf flush in defoliation saplings from all taxa on low fertility habitats, and across all saplings, change in wood P pools correlated with P allocated to new leaf production. In three of four taxa on the low fertility montane habitat, there was significant depletion in wood N pools, suggesting that N remobilization may occur in tropical montane forests where N-limitation is thought to be stronger than in lowland forests. By demonstrating that wood P pools are dynamic reserves functionally linked to tissue production, this study provides novel insight into nutrient allocation of trees in tropical forests.

INTRODUCTION

Because trees are rooted in the ground, they have a very limited capacity to modify their access to resources. Therefore, plants are under strong selection to store water, sugars, and mineral nutrients needed for growth and metabolism during times when resources are scarce (Chapin *et al.*, 1990). In temperate forests, storage dynamics must be studied in the context of climate seasonality, where winter represents a period of severe resource limitation. Deciduous trees therefore maintain sufficient carbohydrate and mineral nutrients to flush new leaves in the spring. In fact, carbohydrate reserves are sufficient to support multiple leaf flushes (Hoch *et al.*, 2003), and nitrogen stores in wood and roots are the primarily source of nitrogen in reflushed leaves in many species (Millard & Grelet, 2010). Much less is known about how trees in less seasonal environments, such as tropical forest, use stored reserves to mitigate periods of resource scarcity. The extent to which tropical trees allocate nutrient, carbohydrate, and water reserves to

storage strongly influences our understanding of the factors that limit their productivity and ability to respond to global change players known to influence tropical forests such as nitrogen deposition (Hietz *et al.*, 2011) and prolonged periods of drought (Phillips *et al.*, 2009).

While high temperatures and abundant precipitation in lowland and lower montane moist tropical forest is conducive to plant productivity, tropical forests experience persistent resource limitation that is expected to select for reserve formation. Light limitation in the understory of tropical forests can be severe, with as little as 1% of photosynthetically active radiation present above the canopy reaching the forest floor (Chazdon & Fetcher, 1984). Consequently, trees adapted to low light conditions maintain large reserves of nonstructural carbohydrates both as seedlings (Myers & Kitajima, 2007) and saplings (Poorter & Kitajima, 2007) to survive damage and defoliation. Additionally, the majority of tropical forests have a distinct wet and dry season, so storage dynamics are also dictated to a certain extent by seasonality. In Panama, carbohydrate dynamics of woody biomass track seasonality, yet saplings (Tissue & Wright, 1995) and adult trees (Würth *et al.*, 2005) also maintain large carbohydrate reserves in branches and wood year round. Plants in seasonally dry tropical forests are also known to maintain significant trunk water storage to mitigate water stress in the dry season (Borchert, 1994).

The importance of soil nutrient limitation in selecting for storage reserves is less frequently acknowledged. The same climate factors that enable year round plant growth also promote weathering and leaching of soils, making many tropical forests severely depleted in the availability of rock derived nutrients, most notably phosphorus (P) (Walker & Syers, 1976). Long term nutrient addition experiments show that tropical forest productivity is limited by the availability of one or more soil nutrients (Vitousek, 1984; Wright *et al.*, 2011), with nitrogen (N) limitation becoming important in montane forests (Tanner *et al.*, 1998). Despite expectations of

N vs. P limitation over broad spatial gradients, at local scales, co-occurring tropical tree species vary enormously in N and P allocation in both low and high fertility environments (Townsend *et al.*, 2007; Heineman *et al.*, 2016) and in both lowland and montane forests (Dalling *et al.*, 2016). Consequently, we should expect storage allocation and remobilization capacity to vary not only among habitats but also among co-occurring taxa.

Insights into the dynamics of nutrient storage in tropical forests can be gained by examining tissue nutrient concentrations in nutrient addition experiments and along soil fertility gradients. Experimental addition of N and P tends to result in greater increases in P compared to N in foliar biomass (Ostertag, 2010), although species also differ in foliar responses to nutrient addition (Mayor *et al.*, 2014). The stoichiometric responses to nutrient addition also tend to be more pronounced in stem biomass compared to fresh foliar biomass (Harrington *et al.*, 2001; Schreeg *et al.*, 2014). A survey of wood and leaf nutrients over soil gradients show that wood nutrients are more sensitive to variability in soil P availability than soil N availability, and that proportionally more P accumulates in wood at high concentrations of foliar P (Heineman *et al.*, 2016). These differences may arise from differences among elements in the chemical form or anatomical location of storage. In the case of nitrogen, where allocation processes are best understood, organically bound molecules such as amino acids are used for storage (Pate, 1973). Therefore, nitrogen storage may incur an additional carbon cost (Millard, 1988) that could prevent excess nitrogen accumulation. In contrast, phosphorus may be stored inorganically in vacuoles (Sinclair & Vadez, 2002). Therefore, we might expect the size, mobility, and interspecific variation in plant storage to differ between nitrogen and phosphorus by virtue of their biochemistry and the degree to which they limit plant growth.

Here, we evaluated if trees are capable of mobilizing N and P from stems in response to an experimentally imposed stress. We conducted a sapling defoliation experiment in Panama, which included four tree taxa sampled in high and low fertility habitats in both lowland and montane forest sites. We measured N and P concentrations in foliar and woody biomass before experimental defoliation and in woody biomass again after the creation of new leaves post-defoliation to determine the flux of nutrients from wood reserves. We hypothesized that if woody biomass is an important mobile nutrient repository, then change in wood P concentrations should correlate with the P allocated to leaf area created in response to defoliation. Second, if mobilization of N and P from wood reserves is more important where nutrient supply is low, then plants should remobilize more N and P in response to defoliation on low fertility relative to high fertility soils. With respect to differences among elements, we hypothesized that if the importance of N-limitation increases with elevation in tropical forests, then the magnitude of wood N remobilization should be greater in montane forests than in lowland forests. Furthermore, if N-fixing species have access to an immediately available N-supply from their symbionts, then legumes should remobilize less N from wood compared to other taxa.

MATERIALS AND METHODS

Study sites and focal taxa

Saplings included in this study were sampled from high and low fertility habitats selected from networks of lowland and montane permanent forest plots (Table 5.1). Montane sites were located within the Fortuna Forest Reserve in western Panama. Fortuna encompasses 19,500 ha of old growth pre-montane and lower montane wet tropical forest ranging 800-1500 m asl. Average temperature in the area is 19°C (Cavelier *et al.*, 1997) and mean annual rainfall ranges 4500-6500 mm yr^{-1} depending on orographic position (Prada et al. *in review*). Twelve 1-ha permanent

forest plots have been established at Fortuna in which all the trees > 5 cm have been measured and identified to species (Prada *et al., in review*; see Figure 1 Chapter 3 for map). Soil pH, inorganic N, and available ("resin") P were measured in 13 locations per plot according the methods outlined in Andersen *et al.*, (2010) and Prada *et al*., *in review* (Table 5.1). From these 12 plots we selected two sites, Honda A and Pinola, to represent low and high fertility, respectively, in this experiment. Honda A and Pinola are located 2.2 km apart but differ three-fold in soil N and four-fold in soil P availability (Table 5.1). In two years of concurrent rainfall collection (2012-2014), Pinola received over 1000 mm less rain annually than HondaA. However, both sites receive > 4000 mm rainfall/year and average > 100 mm rainfall per month during the dry season (Table 5.1). Both sites are highly diverse, containing > 100 species/ha, and also have high stem density of ~1500 trees > 5 cm DBH per ha (Prada *et al., in review*; Table 5.1).

At Fortuna, we selected four focal species present at both sites: *Osteophloeum platyspermum* (Myrisiticaceae), *Faramea multiflora* (Rubiaceae), *Guarea glabra* (Meliaceae), and *Inga alba* (Fabaceae). Species were chosen to maximize contrast in life history characteristics: *O. platyspermum* is a canopy emergent species in the nutmeg family with simple leaves and monopodial branching. *F. multiflora* is an understory shrub with simple leaves and monopodial branching. *G. glabra* is a mid canopy species with compound leaves with indeterminate leaflet growth (Steingraeber & Fisher, 1986). *I. alba* is mid canopy species with compound leaves and shrub-like architecture. Like many legumes, *Inga* produce root nodules and are known facultatively fix nitrogen (Barron *et al.*, 2011).

We selected lowland sites from a network of over 50 1-ha plots established in the Panama Canal watershed by the Center for Tropical Forest Science, in which all stems > 1 cm DBH have

been measured and identified to species (Pyke *et al.*, 2001). These plots represent seasonally moist mature secondary tropical forest (Pyke *et al.*, 2001). The average temperature in the region is 27°C (Windsor, 1990) and average rainfall varies across the Isthmus of Panama from 1700 mm yr^{-1} on the Pacific coast to 3400 mm yr^{-1} on the Caribbean coast (Turner & Engelbrecht, 2011). For the majority of these sites, soil nutrients were determined using the same protocol as the Fortuna plots (Turner & Engelbrecht, 2011; Condit *et al.*, 2013).

High and low fertility sites were chosen to maximize the contrast in soil nutrient availability among locations where taxa in the same family or genus as the four focal species selected at Fortuna were available for sampling. Because lowland sites have much lower average diversity and stem density than the montane sites (Table 5.1), it was not possible to find a single low and high fertility site where all four taxa were present. We chose two high fertility (P13 and P24) and two low fertility sites (P06 and P26), which span threefold variation in soil N and 65 fold folair in soil P. Annual rainfall of focal sites span 2150-2590 mm yr^{-1} (Table 5.1). P13 is located in the Barro Colorado Island Nature Monument on the Buena Vista peninsula, P06 is located in Parque Soberania on Pipeline Road, P24 is located in Parque Soberania near the Venta de Cruces, and P26 is located in the Rio Paja watershed (see Figure 4.1 in Chapter 4).

We chose to include lowland taxa that were closely related to the species sampled in Fortuna (Figure 5.1). Because there is strong taxonomic signal in foliar and wood nutrient content in tropical tree species (Heineman *et al.,* 2016), congeneric and confamilial species are likely to be similar enough in nutrient allocation to provide relevant contrast between montane and lowland forests. The lowland nutmeg analog to *O. platyspermum* was the confamilial species *Virola sebifera* (Myristicaceae), which was sampled at P24 (high fertility) and P25 (low fertility). We sampled two species of *Faramea* in the Panama Canal watershed including

Faramea occidentalis at P13 (high fertility) and *Faramea luteovirens* at P06 (low fertility). We also sampled two species of *Guarea* including *Guarea guidonia* at P24 (high fertility) and *Guarea* "fuzzy" at P06 (low fertility). We sampled two facultatively N fixing legumes: *Inga nobilis* at P13 (high fertility) and *Tachigali versicolor* at P25 (low fertility). For the most part, lowland focal taxa were very similar to their montane analog taxa in leaf morphology, branching architecture, and life history (Figure 5.1). However, the canopy emergent tree *T. versicolor* is distinct from *Inga* species in its adult stature and monocarpic life form, giving it the nickname "suicide tree" (Foster, 1977). Also, *T. versicolor* saplings typically create large branch-like compound leaves which extend from a single primarily stem, in contrast to *Inga* which exhibit higher branching order as saplings (Figure 5.1). While *T. versicolor* is in a non-fixing legume clade Caesalpinioideae and may use a different N-fixing symbionts than those found in the nodules of *Inga* and other Mimosoideae (Parker, 2000), this species has been shown to fix nitrogen in Panamanian forests (Batterman *et al.*, 2013).

Selection of saplings

The defoliation experiment was carried out in five stages: 1) selection of focal saplings, 2) monitoring of saplings for seasonal leaf flush, 3) defoliation of experimental trees and sampling of initial wood and leaf tissue, 4) monitoring of saplings for the re-flushing of leaves in response to defoliation treatment, and 5) resampling of wood tissue and destructive harvest of a subset of saplings (Figure 5.2). From July-October 2013, we selected 13-25 saplings per taxon per habitat (lowland low fertility, lowland high fertility, montane low fertility, montane high fertility) for a total of 286 saplings. To ensure that saplings grew in soil environments similar to the plot-based soil measurements, we sampled individuals inside or within 100 m of the 1-ha plots listed in Table 5.1. We selected saplings that were short enough for leaves to be removed

manually (< ~ 3 m), but taller than 0.5 m at the time of selection. We avoided selecting individuals in tree fall gaps, with severe damage or disease, or individuals that had clear evidence of previous resprouting. After selection, we measured sapling characteristics including diameter at 10 cm and 50 cm height, height to the tallest leaf, and leaf number (Table 5.2). We measured leaf area for five leaves per individual for five trees per species using a LI-3100C leaf area meter (LI-COR, Lincoln, NE) to determine the average leaf area of each species, which was then used to calculate total leaf area for each individual sapling. For the same five individuals per species, we measured the dry mass of leaves to calculate species mean leaf area per unit leaf mass (specific leaf area (SLA cm^2 g^{-1}). We measured canopy openness as a metric of sapling light environment from hemispherical photos taken at 1.3 m above the base of each focal sapling. Focal individuals were carefully pushed away from the frame of the picture to ensure no self-shading of the image. Hemispherical photos were analyzed using Gap Light Analyzer version 2.0 (Frazer *et al.*, 1999).

After selection, the leaf production of focal samplings was evaluated once per month (October-June) for montane saplings and once every two-three months for lowland saplings (October, January, April, June). The purpose of tracking leaf production was to detect the seasonal flush of leaves after the transition from dry season to the wet season, so that the defoliation treatment could be started after saplings had used nutrients allocated to seasonal reserves. Studies of sapling phenology on Barro Colorado Island in Panama suggest that peak leaf production is in May, during the transition from the dry to the wet season (Barone, 1998). Patterns of canopy litterfall in Fortuna also suggest seasonality in leaf production despite high year round rainfall (Heineman *et al.*, 2015). We tracked leaf production by marking new leaves with a color that corresponded to each month with water resistant paint. Leaf area production

was calculated by multiplying leaf number by the average species leaf area. By June 2014, nearly all the lowland saplings had demonstrated a significant leaf flush, which was our cue to start the defoliation experiment. The same was not true for saplings at Fortuna, which put leaves on gradually throughout the dry season. We decided for consistency that we would defoliate all individuals in the month of June, while making the assumption that the Fortuna individuals were aseasonal in their phenology.

Defoliation procedure

Before defoliation, we stratified saplings of each species and habitat by size and light environment and assigned two-thirds of the individuals to the "defoliation group", and one-third (but no fewer than five individuals) to the "control group". The control group was included to 1) test if defoliated individuals created more leaves in response to our treatment than would be expected based on phenology and 2) to determine if the tissue collection itself caused sapling mortality. From both control and defoliated individuals, we sampled 2 cm in diameter leaf discs from the three youngest fully expanded leaves for each species before defoliation. We also sampled wood tissue in control and experimental saplings prior to defoliation by collecting wood shavings generated by drilling the stem using a cordless electric drill (DeWalt DCD780B) with 1/8" drill bit which yielded ~30-60 mg of dry wood per sample. We drilled four points on the stem beginning 10 cm below the branching point spaced 10 cm apart. In cases, where the saplings were < 1 cm diameter at 50 cm, I used a 1/16" drill bit. We scraped bark off the stem at drilling points prior to incision so that samples were not affected by higher nutrient bark material. Wood and leaf samples were stored on ice until they could be brought transported to an oven where they were dried at 60 C for 72 hours. After wood and leaf tissues were collected, we

removed 75% of leaves from defoliated individuals. For species with compound leaves, we removed 75% of leaflets, leaving rachii and petioles intact.

After defoliation, we returned to each sapling every three weeks to monitor leaf production. Once the majority of defoliated trees for a given a taxon and site demonstrated a discrete leaf flush and had no remaining emerging buds (average time 4.5 months, range 3.5 – 5 months) we re-sampled wood from both control and experimental individuals by drilling the stem at points in between the initial drill holes. We also harvested the new leaves that had been created post-defoliation and measured the leaf area using the LI-COR leaf area meter. For control individuals, we re-measured stem diameter and finalized our records of leaf production so that we would have a full year of sapling growth data for each species/habitat combination. We destructively harvested 50% of control and defoliated individuals to determine the biomass of each plant organ. We weighed the fresh biomass of stems, branches, roots, and leaves for each harvested sapling. We dried three leaves and a stem segment for each sapling to determine the correct for water content of each sapling.

During the second (post-defoliation) monitoring period, 9 of 30 saplings of *O. platyspermum* at Fortuna died from trees falls or herbivory from beetle larvae, and 13 of 17 individuals of *F. luteovirens* in the lowland forests experienced intense herbivory by leaf cutter ants, resulting in the near complete defoliation of both control and experimental individuals. We present the number of individuals selected and final sapling number in Table 5.2. Overall, 9% of saplings died between the selection individuals July-Oct 2013 to the end of the experiment in Nov 2014. With the exception of *O. platyspermum,* the majority of deaths were in tree fall gaps and mortality rates were similar for the monitoring periods prior to defoliation (Oct 2013-June 2014) and after defoliation (June 2014-November 2014), indicating that sampling of stem tissue

did not markedly affect sampling mortality over the duration of the experiment. Our final dataset included 257 saplings.

Chemical analysis

Nitrogen concentrations in leaf and wood tissues were tested on a Costech Elemental Analyzer (Valencia, CA) for samples analyzed at the University of Illinois. To prepare wood and leaf material for P analysis, samples were dry ashed at 550°C for 2 hours and the ash dissolved in 1 M HNO_3 (Karla, 1998). Base cations and P in leaves were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) on an Optima 2000 DV (PerkinElmer, Waltham, MA). Because our drilling method yielded only a small quantity of wood tissue, P concentrations were below the ICP-OES detection limit and were instead analyzed via automated molybdate colorimetry using the Lachat Quickchem 8500 (Hach Ltd., Loveland, CO). We included certified reference samples (NIST 1515, apple leaves) and internal laboratory control standards in all analyses.

Statistical analysis

We evaluated the effect of taxon and fertility on wood and leaf N and P concentrations using two-way ANOVA models including the taxon × fertility interaction on wood N, wood P, leaf N, and leaf P. We fit models separately for lowland and montane sites because soil type comparisons were intraspecific at the montane and interspecific at the lowland site. Wood and leaf nutrient values included in these analyses were the "initial" samples collected from control and defoliated individuals immediately prior to leaf removal of defoliated saplings (time 1; Figure 5.2).

To determine if defoliated saplings remobilized a significant fraction of P and N from wood stores, we calculated "remobilization" or the percent difference between wood N and wood

P concentrations for each defoliated sapling at the time of defoliation (Conc. Initial) from the concentration after leaf reflush (Conc. Final):

Remobilization (%) = (Wood Conc. Final - Wood Conc. Initial)/ Wood Conc. Initial × 100

Negative remobilization values signify that wood nutrient reserves had been depleted in responses after defoliation and positive values signify that there was an increase in wood nutrient concentrations from time one to time two. We fit two-way ANOVA models testing the interaction effect of fertility and taxa on N and P remobilization, however, the interaction was not significant in any models so we present models without the interaction. Models were fit separately for lowland and montane sites.

To evaluate if remobilization was proportional to leaf creation, we calculated the total quantity of N and P remobilized from stem biomass and the total N and P allocated to new leaf production as follows:

Total Remobilized (g) = (Wood Conc. Final - Wood Conc. Initial)/100 × Dry Stem Biomass

Total in New Leaves (g) = Leaf $Area_{new}$ * Foliar Conc. Initial/100 × (1/SLA)

where Leaf $Area_{new}$ is the leaf area produced from time 1 to time 2 (Figure 2). We used multiple regression models to determine the relationship between total N and P remobilized and total N and P in new leaves. We included both control and defoliated individuals that had been harvested (N = 155 saplings) and tested for the effects of treatment, taxa, and fertility. Total N and P in new leaves were square root transformed to meet distributional assumptions.

RESULTS

Leaf creation in response to defoliation

The leaf creation response of saplings after defoliation differed among taxa and habitats. For the majority of taxon/habitat combinations, leaf area created by defoliated individuals did not

exceed leaf creation by control individuals over the same period of time (Figure 5.3). The contrast between treatment groups was the greatest on the lowland low fertility soil where defoliated individuals created more leaf area over the experimental period than control individuals for all taxa but *Faramea*, which experienced intense persistent herbivory by leaf cutter ants (Figure 5.3). On high fertility lowland soils, defoliated individuals of compound leaved taxa (legumes and *Guarea*) created more leaf area than control individuals, while defoliated Nutmeg and *Faramea* individuals created roughly equal amounts of leaf area on average relative to control individuals. In the montane sites, where leaf flushing did not occur prior to defoliation at the transition from the dry to wet season, there was higher overall leaf production in both control and defoliated individuals compared to lowland taxa, and defoliated saplings created more leaf area than control individuals in only one case: *O. platyspermum* on high fertility soil. Despite a lack of contrast relative to control individuals, defoliated individuals refoliated a substantial amount of removed leaf area on average in both lowland (23%) and montane (41%) forest. Only 13 of 155 defoliated saplings created no leaves or buds within 5 months of the defoliation procedure compared to 30 of 110 control saplings over the same time period.

Natural variation of N and P in wood and leaves

For the four species sampled on low and high fertility soils at Fortuna, concentrations of N and P in foliar and woody biomass varied substantially within and among soil habitats. We a found a marginally significant species x fertility interaction for models predicting variation in foliar N ($F_{3,83} = 2.7$, $P = 0.051$) and foliar P ($F_{3,83} = 2.6$, $P = 0.060$, Table 3, Figure 5.4), and the main effects of species and fertility were significant for both foliar N and foliar P (Table 5.3). Foliar N was significantly greater on the high fertility soil than the low fertility soil for all

species but *O. platyspermum*, and foliar P was significantly higher on the high fertility soil for all species but *G. glabra* (Figure 5.4). Species and habitat together explained the vast majority of variance in foliar N ($r^2 = 0.70$), whereas the majority of variance in foliar P was not explained by measured variables ($r^2 = 0.27$).

In contrast to foliar nutrients, which displayed qualitatively similar patterns of variation for N and P, the relative importance of site and species differed between wood N and wood P. Wood N concentrations did not differ among soil habitats ($F_{3,83} = 2.9$, $P = 0.093$), but did vary significantly among species ($F_{3,83} = 27.0$, $P < 0.001$; Table 5.3; Figure 5.4). In contrast, the effect of species on wood P was only marginally significant ($F_{3,83} = 2.5$, $P = 0.060$), while wood P did differ significantly among habitats ($F_{3,83} = 25.1$, $P < 0.001$). The magnitude of the difference in wood P concentrations among sites was striking, as wood P concentrations were 36% higher on average for saplings sampled on the high fertility habitat than on the low fertility habitat. Similar to foliar nutrients, species and site together explained a larger proportion of variation in wood N ($r^2 = 0.47$) compared to wood P ($r^2 = 0.25$).

With the exception of *Virola sebifera*, we could not evaluate intraspecific effects of nutrient availability on tissue chemistry in lowland species because our soil type comparisons were limited to confamilial and conspecific pairs. For lowland species, we tested for a fertility × taxon interaction to evaluate if patterns of variation within species are similar to patterns observed across closely related species. There was a significant taxon x fertility interaction on foliar N ($F_{3,109} = 7.2$, $P < 0.001$), as foliar N was higher on high fertility than low fertility soils for *Faramea*, while foliar N was lower on the high fertility soil for *Guarea* and Legume taxa (Figure 4). In models evaluating foliar P, the main effects of taxon ($F_{3,109} = 148.8$, $P < 0.001$) and fertility ($F_{3,109} = 19.7$, $P < 0.001$) were significant, as foliar P was higher on high fertility soil

than low fertility soil in all four taxa (Figure 5.4). Taxon and habitat explained a large proportion of variance in both foliar N ($r^2 = 0.78$) and foliar P ($r^2 = 0.81$). The significant fertility × taxon interaction effect ($F_{3,109} = 7.0$, $P < 0.001$) on wood N indicates that wood N allocation differed among soil habitats, but this differences was not in the direction expected: wood N was higher on low fertility soil for *Faramea* and Legume taxa, and did not differ between soil habitats in the other two taxa. As expected, wood P concentrations were higher on high fertility compared to low fertility soils for three of four species (Figure 5.4), and there was a significant fertility × taxon interaction effect ($F_{3,109} = 14.1$, $P < 0.001$), driven by the extremely high concentrations of P in the wood of taxa sampled at the site with the highest soil available P in this study, P24 (Figure 5). For *V. sebifera*, the only lowland taxon for which intraspecific comparisons were possible, mean wood P of individuals sampled on P24 (0.091%) was over six times greater than the mean wood P of individuals sampled on the low fertility habitat P26 (0.014%). Furthermore, the mean concentration of P did not differ between wood and leaves for *V. sebifera* individuals sampled on P24 (Figure 5.4), while mean wood P concentrations were five times smaller than mean leaf P concentrations for *V. sebifera* individuals sampled on low fertility soil. Fertility and taxa together accounted for more than half of the variation in wood N ($r^2 = 0.58$) and wood P ($r^2 = 0.51$) in lowland saplings.

Remobilization of N and P from wood

The change in wood P concentrations, or P remobilization, was significantly less than zero sampled for all taxa/habitat combinations sampled on low fertility soils (Figure 5.5), indicating that wood P concentrations were depleted over the course of the experiment. The average proportion of wood P remobilized was greater on montane (29.8%) compared to lowland (16%) low fertility forests, perhaps due to greater leaf production at the low fertility montane

habitat between defoliation and harvest (Figure 5.3). On high fertility soils, saplings from compound leaved taxa (Legume and *Guarea*) remobilized a significant fraction of P on average at both lowland and montane forests, while Nutmeg taxa did not change significantly on average in wood P at either high fertility site and *F. occidentalis* sapling increased in wood P on average from time 1 to time 2 (Figure 5.5). Consequently, there was a significant fertility effect on wood P remobilization for models evaluating the effect of fertility and taxa on remobilization in both montane ($F_{1,51}$ = 0.6.2, P = 0.016) and lowland ($F_{1,64}$ = 5.1, P = 0.027) sites.

Wood N remobilization patterns were idiosyncratic across species and habitats. Wood N concentrations of N-fixing Legume species increased from time 1 to time 2 on average for taxa sampled on both high and low fertility soils in lowland forests (Figure 5.5). Consequently there was a significant effect of sapling taxa on wood N remobilization among lowland species ($F_{3,64}$ = 7.2, P < 0.001). N remobilization from wood was most prevalent at the low fertility montane habitat, where average wood N remobilization was significantly less than zero for three of four species (Figure 5.5). However, the effect of fertility was not significant for saplings sampled in the lowland ($F_{1,64}$ = 0.0, P = 0.280) or montane ($F_{1,51}$ = 1.4, P = 0.241) forest.

Across all saplings destructively harvested, there was a significant relationship between the total quantity of P remobilized from stem biomass and the quantity of P allocated to new leaves across species (r^2 = 0.28, df = 1,155, P < 0.001, Figure 5.6). This relationship did not differ between control and defoliated groups, indicating our experimentally imposed stress did not increase tree reliance on P reserves. There was also a significant but weak relationship between wood N remobilization and N allocated to new leaves (r^2 = 0.04, df = 1,155, P = 0.006).

DISCUSSION

Despite widespread evidence that phosphorus (P) limits productivity of tropical trees (Vitousek, 1984), the dynamics of P storage and remobilization are poorly understood in tropical trees. Our results demonstrate that wood contains a dynamic pool of P that reflects both P supply from the soil and demand for P allocation to new tissue growth. The strong sensitivity of wood P to soil P availability is in line with previous observations from tropical forests which have shown a fourfold increase in wood P in response to P addition in Hawaii (Harrington *et al.*, 2001) and an eightfold increase in community mean wood P across a soil fertility gradient in Panama (Heineman *et al.*, 2016). Foliar P also increased from low to high fertility habitats within species at Fortuna, and among closely related taxa in the Panama Canal watershed, supporting previous observations that P may accumulate in the foliar tissues of tropical plants (Ostertag, 2010), although the proportional effect of soil fertility on wood P concentrations was much greater than the effect on leaf P concentrations. In particular, the enormous discrepancy in wood P concentrations between the Nutmeg and *Guarea* taxa among lowland sites, where the contrast in soil P concentrations was extreme, indicated that wood P concentrations are much less constrained by taxonomically prescribed allocation strategies than fresh foliar nutrient concentrations. Not only did natural variation in wood P reflect supply of P from the soil, the total change in stem phosphorus pool correlated with the allocation of P to new leaf tissue, indicating that there is a functional link between phosphorus remobilization and tree growth. Wood P concentrations significantly declined post defoliation in all four taxa sampled on low fertility soils, whereas wood P declined in only two of four species declined sampled on high fertility soils. This soil fertility effect supports our hypothesis that remobilization from storage reserves is more important where soil nutrient supply is low. It is well established that trees

remobilize a greater proportion of P from senesced leaves where soil P availability is scarce (Vitousek, 1984; Kitayama *et al.*, 2004), however, this finding provides novel insight into the importance of wood as a repository for mobile P reserves.

While phosphorus mobilization appeared to be important at both lowland and montane sites, we found support for our hypothesis that wood N remobilization should be more important in montane forest. We observed significant average declines in wood N concentrations after refoliation for three of four species sampled at the low fertility site at Fortuna, where a long term fertilization experiment provides evidence of N-limitation productivity (Adamek *et al.,* 2009; Heineman *et al.,* 2015). Furthermore, there was a significant effect of fertility on foliar N at Fortuna, which parallels with species patterns of foliar N within understory palm communities at this site (Andersen *et al.,* 2012). Despite significant differences in foliar N, there was no effect of soil fertility on wood N, consistent with findings from fertilization experiments that wood nitrogen status is less sensitive to nutrient addition than wood phosphorus (Harrington *et al.,* 2001; Mo *et al.,* 2015).

While we observed general trends in nutrient remobilization dynamics, N and P allocation and remobilization patterns differed widely among the four taxonomic groups included in this study. Intraspecific responses in wood and leaf N and P concentrations to variation in soil resources differed among species, similar to the varied species responses of foliar chemistry to nutrient addition observed in a Panamanian fertilization experiment (Mayor *et al.*, 2014). Notably, legume taxa in low fertility soils increase in wood N concentrations on average post defoliation, suggesting that N-fixation may have been upregulated in response to stress. In addition, both taxa with compound leaves (Legume and *Guarea*) species responded more strongly to defoliation in terms of both P remobilization and leaf area creation than simple

leaved taxa. Variation among species in nutrient allocation and acquisition strategies is implicated as an important driver of multiple nutrient limitation in tropical forests (Townsend *et al.*, 2011; Dalling *et al.*, 2015). The results of this experiment indicate that nutrient storage and remobilization could be important for comparing nutrient use strategies among tropical tree species.

While this experiment provides several interesting insights into the nature of N and P dynamics in tropical plants, there are a number of methodological concerns that could complicate the interpretation of results. First, the majority of the habitat/taxa pairs evaluated, defoliated individuals did not create significantly more leaf area over the study period relative to control saplings. The intended effect of defoliation was to induce saplings to produce more leaf tissue, and, thereby, determine if N and P remobilization of woody biomass occurs when there is a sudden demand for foliar tissue production. Given that both control and defoliated plants produced similar numbers of leaves, the decline in wood P in defoliated individuals likely reflected seasonal reserve use rather than the emergency reserve usage. Perhaps our defoliation treatment would have induced a greater leaf area and nutrient remobilization response if, rather than removing 75% of leaf area, we had clipped the stem (Poorter *et al.*, 2010; Millard *et al.*, 2001), or serially defoliated saplings and measured declines in wood nutrient concentrations over a multiple periods of time (Wills *et al.*, 2004). However, it is unclear if more severe defoliation treatments would have killed focal saplings and prevented us from resampling wood tissues at a future time point, which would have further limited inference by reducing sample size.

Conclusions

Despite its limitations, this study provides novel evidence that wood represents a dynamic storage repository of P that may be critical for alleviating P-limitation of plant growth

on tropical soils where P is often scarce. Further, the variation in size of wood P store and their usage by tropical saplings among taxa and soil fertility habitats indicate that storage is an important functional attribute that may influence species distributions across edaphically heterogeneous tropical landscapes.

LITERATURE CITED

Adamek M, Corre MD, Hölscher D. **2009**. Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, panama. *Journal of Tropical Ecology* **25**, 637-647.

Andersen KM, Turner BL, Dalling JW. **2010**. Soil-based habitat partitioning in understorey palms in lower montane tropical forests. *Journal of Biogeography* **37**, 278-292.

Barone JA. **1998**. Effects of light availability and rainfall on leaf production in a moist tropical forest in central panama. *Journal of Tropical Ecology* **14**, 309-321.

Barron AR, Purves DW, Hedin LO. **2011**. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. *Oecologia* **165**, 511-520.

Batterman SA, Hedin LO, Van Breugel M, Ransijn J, Craven DJ, Hall JS. **2013**. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **502**, 224-227.

Borchert R. **1994**. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology*, 1437-1449.

Cavelier J, Jaramillo M, Solis D, de León D. **1997**. Water balance and nutrient inputs in bulk precipitation in tropical montane cloud forest in panama. *Journal of Hydrology* **193**, 83-96.

Chapin FS, Schulze E-D, Mooney HA. **1990**. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* **21**, 423-447.

Chazdon R, Fetcher N. 1984. Light environments of tropical forests *Physiological ecology of plants of the wet tropics* (pp. 27-36): Springer.

Condit R, Engelbrecht BMJ, Pino D, Pérez R, Turner BL. **2013**. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences* **110**, 5064-5068.

Dalling JW, Heineman K, González G, Ostertag R. Geographic, environmental and biotic sources of variation in the nutrient relations of tropical montane forests.

Dalling JW, Heineman K, Lopez OR, Wright SJ, Turner BL. 2016. Nutrient availability in tropical rain forests: The paradigm of phosphorus limitation *Tropical tree physiology* (pp. 261-273): Springer.

Foster RB. **1977**. Tachigalia versicolor is a suicidal neotropical tree.

Frazer GW, Canham C, Lertzman K. **1999**. Gap light analyzer (gla), version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. *Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York* **36**.

Fyllas NM, Patiño S, Baker TR, Bielefeld Nardoto G, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna V, Mercado LM et al. 2009. Basin-wide variations in foliar properties of amazonian forest: Phylogeny, soils and climate. *Biogeosciences* **6**, 2677-2708.

Harrington RA, Fownes JH, Vitousek PM. **2001**. Production and resource use efficiencies in n- and p-limited tropical forests: A comparison of responses to long-term fertilization. *Ecosystems* **4**, 646-657.

Heineman KD, Caballero P, Morris A, Velasquez C, Serrano K, Ramos N, Gonzalez J, Mayorga L, Corre MD, Dalling JW. **2015**. Variation in canopy litterfall along a precipitation and soil fertility gradient in a panamanian lower montane forest. *Biotropica* **47**, 300-309.

Heineman KD, Turner BL, Dalling JW. **2016**. Variation in wood nutrients along a tropical soil fertility gradient. *New Phytologist*.

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ. **2011**. Long-term change in the nitrogen cycle of tropical forests. *Science* **334**, 664-666.

Hoch G, Richter A, Körner C. **2003**. Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* **26**, 1067-1081.

Karla YP. (1998). Handbook of reference methods for plant analysis: CRC Press, Boca Raton, FL.

Kitayama K, Aiba S-I, Takyu M, Majalap N, Wagai R. **2004**. Soil phosphorus fractionation and phosphorus-use efficiency of a bornean tropical montane rain forest during soil aging with podozolization. *Ecosystems* **7**, 259-274.

Martin AR, Erickson DL, Kress WJ, Thomas SC. **2014**. Wood nitrogen concentrations in tropical trees: Phylogenetic patterns and ecological correlates. *New Phytologist* **204**, 484-495.

Mayor JR, Wright SJ, Turner BL. **2014**. Species-specific responses of foliar nutrients to long-term nitrogen and phosphorus additions in a lowland tropical forest. *Journal of Ecology* **102**, 36-44.

Millard P. **1988**. The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment* **11**, 1-8.

Millard P, Grelet G-a. **2010**. Nitrogen storage and remobilization by trees: Ecophysiological relevance in a changing world. *Tree Physiology* **30**, 1083-1095.

Millard P, Hester A, Wendler R, Baillie G. **2001**. Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Functional Ecology* **15**, 535-543.

Myers JA, Kitajima K. **2007**. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology* **95**, 383-395.

Ostertag R. **2010**. Foliar nitrogen and phosphorus accumulation responses after fertilization: An example from nutrient-limited hawaiian forests. *Plant and Soil* **334**, 85-98.

Parker MA. **2000**. Divergent bradyrhizobium symbionts on tachigali versicolor from barro colorado island, panama. *Systematic and applied microbiology* **23**, 585-590.

Pate J. **1973**. Uptake, assimilation and transport of nitrogen compounds by plants. *Soil Biology and Biochemistry* **5**, 109-119.

Phillips OL, Aragão LE, Lewis SL, Fisher JB, Lloyd J, López-González G, Malhi Y, Monteagudo A, Peacock J, Quesada CA. **2009**. Drought sensitivity of the amazon rainforest. *Science* **323**, 1344-1347.

Poorter L, Kitajima K. **2007**. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology* **88**, 1000-1011.

Poorter L, Kitajima K, Mercado P, Chubiña J, Melgar I, Prins HH. **2010**. Resprouting as a persistence strategy of tropical forest trees: Relations with carbohydrate storage and shade tolerance. *Ecology* **91**, 2613-2627.

Pyke CR, Condit R, Aguilar S, Lao S. **2001**. Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science* **12**, 553-566.

Schreeg LA, Santiago LS, Wright SJ, Turner BL. **2014**. Stem, root, and older leaf n:P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology* **95**, 2062-2068.

Sinclair TR, Vadez V. **2002**. Physiological traits for crop yield improvement in low n and p environments. *Plant and Soil* **245**, 1-15.

Steingraeber DA, Fisher JB. **1986**. Indeterminate growth of leaves in guarea (meliaceae): A twig analogue. *American Journal of Botany*, 852-862.

Tanner EVJ, Vitousek PM, Cuevas E. **1998**. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**, 10-22.

Tissue D, Wright S. **1995**. Effect of seasonal water availability on phenology and the annual shoot carbohydrate cycle of tropical forest shrubs. *Functional Ecology*, 518-527.

Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. **2007**. Controls over foliar n:P ratios in tropical rain forests. *Ecology* **88**, 107-118.

Townsend AR, Cleveland CC, Houlton BZ, Alden CB, White JW. **2011**. Multi-element regulation of the tropical forest carbon cycle. *Frontiers in Ecology and the Environment* **9**, 9-17.

Turner BL, Engelbrecht BM. **2011**. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**, 297-315.

Vitousek PM. **1984**. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **65**, 285-298.

Walker T, Syers JK. **1976**. The fate of phosphorus during pedogenesis. *Geoderma* **15**, 1-19.

Wills A, Burbidge T, Abbott I. **2004**. Impact of repeated defoliation on jarrah (eucalyptus marginata) saplings. *Australian Forestry* **67**, 194-198.

Windsor DM. **1990**. Climate and moisture variability in a tropical forest: Long-term records from barro colorado island, panama.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE et al. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**, 1616-1625.

Würth MK, Pelaez-Riedl S, Wright SJ, Körner C. **2005**. Non-structural carbohydrate pools in a tropical forest. *Oecologia* **143**, 11-24.

Table 5.1 Site characteristics of permanent 1-ha forest plots where saplings were selected for defoliation. We sampled trees from two low fertility and two high fertility sites in the lowland, seasonally moist forest in the Panama Canal watershed. We sampled trees from one low and one high fertility site in lower montane forest of Fortuna Forest Reserve in western Panama. Soil N and P concentrations were measured from the top 10 cm of soil from 13 locations per plot. Stem density and basal area were calculated from individuals > 5 cm DBH in each plot. Species richness for Fortuna plots include provisional morphospecies (Prada *et al., in review*).

	Lowland (Panama Canal watershed)				Montane (Fortuna Forest Reserve)	
	Low Fertility		High Fertility		Low Fertility	High Fertility
Plot Name	P26	P06	P13	P24	HondaA	Pinola
Latitude (°)	9.076	9.157	9.188	9.124	8.751	8.754
Longitude (°)	-79.787	-79.744	-79.821	-79.677	-82.239	-82.259
Elevation (m)	50	30	55	50	1155	1135
MAP (mm yr^{-1})	2153	2311	2591	2160	6255	4964
Dry Season Rain (mm mo^{-1})	133	147	173	132	430	296
Soil Properties						
pH	3.8	3.9	5.6	5.6	3.2	4.5
Inorganic N (mg kg^{-1})	1.8	2.4	4.6	5.2	3.4	9.3
Resin P (mg kg^{-1})	0.2	1.2	8.4	13.1	0.8	3.5
Forest Properties						
Stem Density (trees ha^{-1})	630	572	698	694	1662	1487
Basal Area (m^2 ha^{-1})	22.2	20.6	41.4	31.2	47.8	48.2
Species Richness	79	85	61	66	167	111

Table 5.2 Size and sample number of sapling taxa in each habitat sampled for our defoliation experiment (ML = montane low fertility; MH = montane high fertility; LL = lowland low fertility; LH = lowland high fertility). For the number of individuals sampled we listed the number selected in October 2013 and the number surviving to the end of the experiment October 2014 in parenthesis. For diameter, height, and leaf area, we list the mean with min and max values parenthesis. For compound leaved species (*) species leaf area was calculated for leaflets rather than entire leaves.

		Number of Individuals		Focal Sapling Measurements			Species Traits	
Taxa	Habitat	Control	Defoliated	Diameter (cm)	Height (m)	Total Leaf Area (cm^2)	SLA ($cm^2\ g^{-1}$)	Leaf Area (cm^2)
Nutmeg								
O. platyspermum	ML	7 (5)	10 (7)	1.28 (0.7-1.9)	1.49 (0.9-2.4)	3032 (1081-5875)	168	47
	MH	5 (5)	8 (4)	1.53 (0.8-2.2)	1.63 (1.1-2.4)	3700 (1457-7426)		
V. sebifera	LL	7 (5)	12 (12)	0.95 (0.6-1.4)	1.60 (1.0-2.3)	3951 (2067-7261)	178	53
	LH	8 (7)	12 (10)	1.05 (0.8-1.4)	1.56 (0.9-1.9)	5751 (3604-9116)		
Faramea								
F. multiflora	ML	7 (7)	10 (9)	0.96 (0.8-1.3)	1.51 (0.9-1.8)	2891 (1404-5772)	203	39
	MH	5 (3)	8 (8)	1.12 (0.8-1.6)	1.57 (1.2-2.4)	4631 (2145-11700)		
F. luteovirens	LL	5 (5)	9 (8)	0.88 (0.4-1.4)	1.72 (1.1-2.5)	3268 (1862-5244)	66	38
F. occidentalis	LH	8 (8)	13 (13)	0.88 (0.6-1.3)	1.67 (1.2-2.2)	3259 (1640-7200	83	40
Guarea								
G. glabra	ML	10 (10)	14 (12)	1.15 (0.6-2.1)	1.85 (1.1-3.0)	2641 (1224-6290)	160	34*
	MH	6 (6)	9 (7)	1.36 (0.9-2.0)	2.12 (1.7-3.1)	4284 (2754-7412)		
G. "fuzzy"	LL	10 (10)	17 (15)	0.92 (0.6-1.6)	1.59 (1.0-2.3)	3728 (2058-9870)	259	49*
G. guidonia	LH	8 (8)	10 (10)	1.07 (0.7-1.6)	1.61 (0.9-2.4)	3847 (1598-7332)	181	47*
Legume								
I. alba	ML	7 (5)	11 (11)	1.36 (0.7-1.8)	1.86 (1.4-2.4)	6054 (2068-12452)	140	44*
	MH	7 (7)	10 (10)	1.26 (1.0-1.6)	1.94 (1.1-2.7)	3898 (1100-7964)		
T. versicolor	LL	6 (6)	10 (9)	1.27 (0.8-1.8)	1.23 (0.7-2.1)	4508 (1680-7620	177	60*
I. nobilis	LH	7 (5)	10 (10)	1.10 (0.7-1.7)	1.79 (1.2-3.2)	4472 (1920-9360)	178	20*

Table 5.3 Analysis of variance table for models evaluating the effect of fertility and taxa on the N and P concentrations of wood and leaf tissues sampled prior to defoliation in saplings included in the defoliation experiment at Fortuna Forest Reserve (montane forest) and the Panama Canal watershed (lowland forest). At the montane forest site, the same four species were sampled on each soil type. At the lowland forest sites, soil contrasts were conducted between congeneric or confamilial pairs

	Montane				Lowland		
	df	*F*	*P*		*df*	*F*	*P*
Foliar N Concentration							
Fertility	1, 83	32.1	< 0.001	Fertility	1, 109	5.9	0.017
Species	3, 83	58.2	< 0.001	Taxa	3, 109	134.1	< 0.001
Fert x Species	3, 83	2.7	0.051	Fert x Taxa	3, 109	7.2	< 0.001
r^2 = 0.70				r^2 =0.78			
Wood N Concentration							
Fertility	1, 83	2.9	0.093	Fertility	1, 109	4.6	0.035
Species	3, 83	27.0	< 0.001	Taxa	3, 109	48.0	< 0.001
Fert x Species	3, 83	1.7	0.168	Fert x Taxa	3, 109	7.0	< 0.001
r^2 = 0.47				r^2 = 0.58			
Foliar P Concentration							
Fertility	1, 83	4.0	0.049	Fertility	1, 109	19.7	< 0.001
Species	3, 83	9.9	< 0.001	Taxa	3, 109	158.8	< 0.001
Fert x Species	3, 83	2.6	0.060	Fert x Taxa	3, 109	1.4	0.248
r^2 = 0.27				r^2 = 0.81			
Wood P Concentration							
Fertility	1, 83	25.1	< 0.001	Fertility	1, 109	52.8	< 0.001
Species	3, 83	2.5	0.060	Taxa	3, 109	11.2	< 0.001
Fert x Species	3, 83	1.2	0.310	Fert x Taxa	3, 109	14.1	< 0.001
r^2 = 0.25				r^2 = 0.51			

Table 5.4 Analysis of variance tables for models evaluating the effect of fertility and taxa on the N and P remobilization in saplings defoliated at Fortuna Forest Reserve (montane forest) and the Panama Canal watershed (lowland forest). At the montane forest site, the same four species were sampled on each soil type. At the lowland forest sites, a given taxa included conspecific (*V. sebifera*), congeneric (*Guarea*, *Faramea*), or confamilial (Fabaceae) individuals.

	Montane				Lowland		
	df	*F*	*P*		*df*	*F*	*P*
Wood N Remobilization							
Fertility	1, 51	1.4	0.241	Fertility	1, 64	0.0	0.280
Species	3, 51	0.3	0.821	Taxa	3, 64	7.2	< 0.001
r^2 = 0.00 *P* = 0.678				r^2 = 0.21; *P* < 0.001			
Wood P Remobilization							
Fertility	1, 51	6.2	0.016	Fertility	1, 64	5.1	0.027
Species	3, 51	2.8	0.049	Taxa	3, 64	1.9	0.128
r^2 = 0.16; *P* = 0.011				r^2 = 0.09; *P* = 0.035			

Figure 5.1 Photos and characteristics of focal tree taxa included in the sapling defoliation experiment. Each taxon was sampled in high and low fertility habitats in lowland forest (Panama Canal watershed) and montane forest (Fortuna Forest Reserve). We sampled one site per fertility group in the montane forest (low fertility = HondaA and high fertility = Pinola) and the same four species were sampled both high and low fertility sites. At the lowland site, we sampled two sites per fertility group and congeners or con-familial pairs were sampled for contrast among soil habitats.

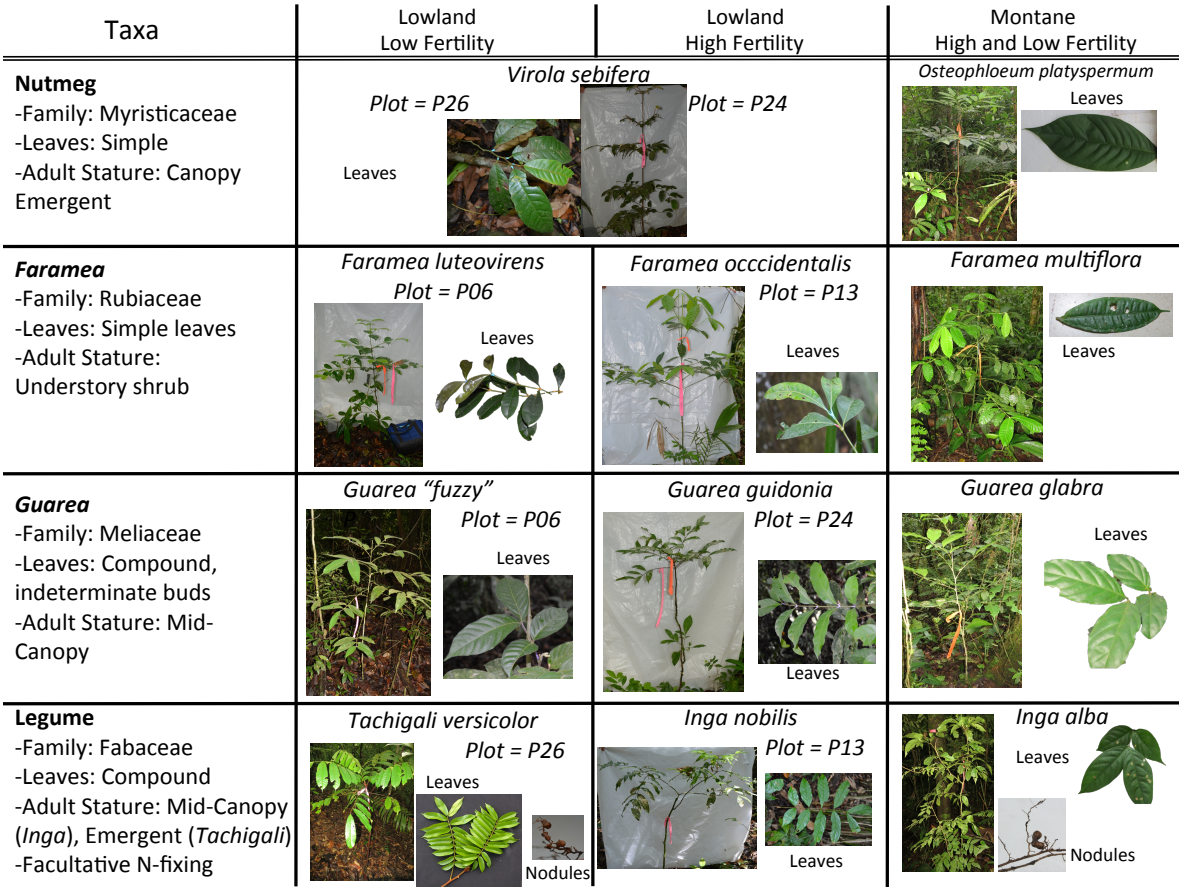


Figure 5.2 Illustration of key events in the sapling defoliation experiment. In June 2014 (Time 1), we sampled initial concentrations of N and P in wood and leaf tissues from saplings prior to defoliation of experimental individuals. In Oct-Nov 2014 (time 2), we resampled wood tissue after the majority of saplings exhibited a discrete leaf reflushing event and then destructively harvested 50% of individuals.

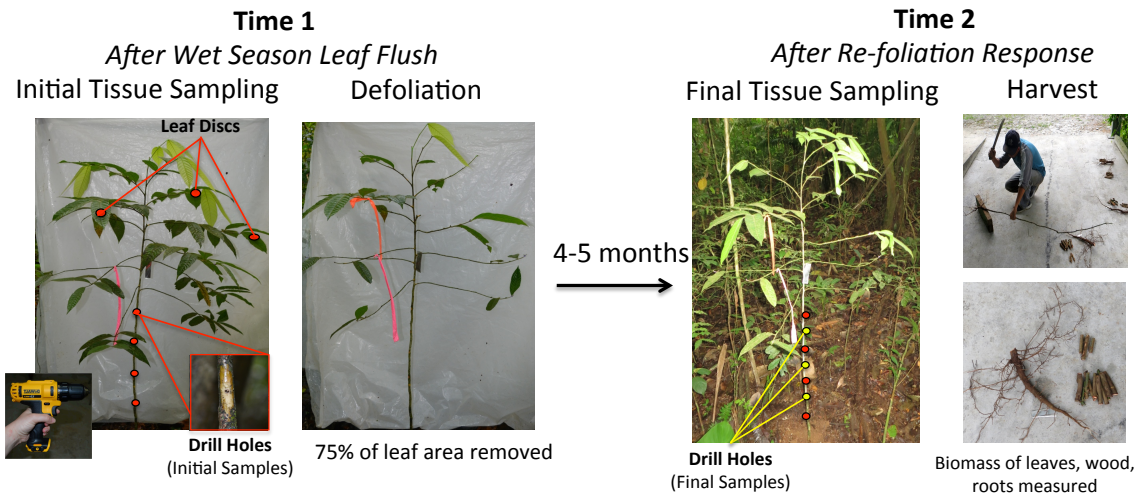


Figure 5.3 Mean percent of initial leaf area reflushed by saplings in control and experimental groups from the beginning of the defoliation treatment (time 1) to the final tissue sampling (time 2) as a percent of initial sapling leaf area. Habitat abbreviations: LL = lowland low fertility, LH = lowland high fertility, ML = montane low fertility, MH = montane high fertility. Y bars represent one standard error

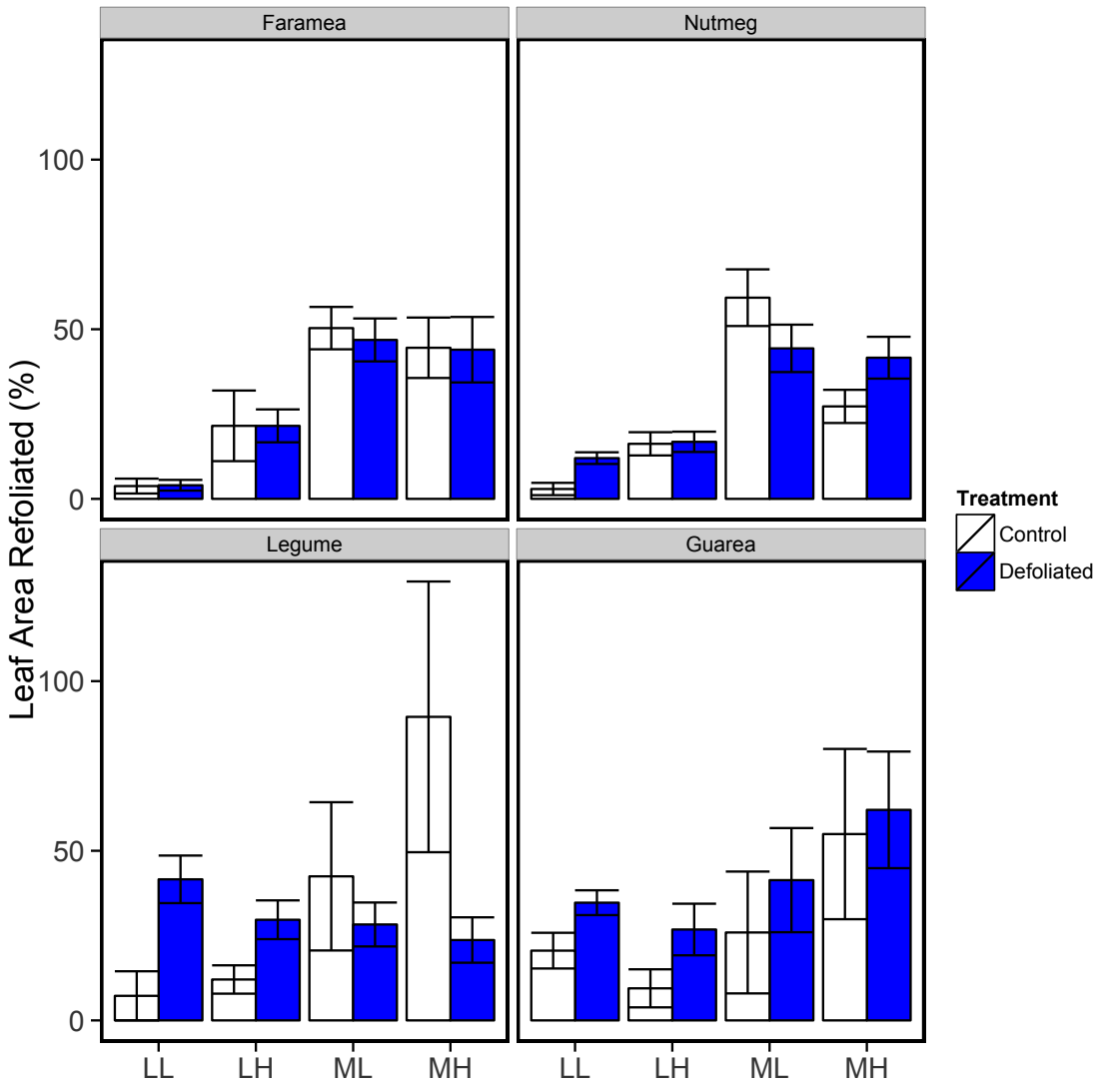


Figure 5.4 Mean initial (pre-defoliation) N and P concentrations of foliar and woody biomass for focal sapling taxa sampled from high and low fertility sites in lowland and montane forests. Soil type comparisons in montane forests are intraspecific comparisons of four species sampled on one high and one low fertility site. Soil type comparisons in lowland taxa represent intraspecific, congeneric, or confamilial comparisons of saplings sampled across two high and two low fertility sites. "*" is shown where N or P concentrations for a taxa differed between soil habitats. Soil N and P concentrations for each site are listed for the top 10 cm of soil in mg kg^{-1}. Y bars represents one standard error.

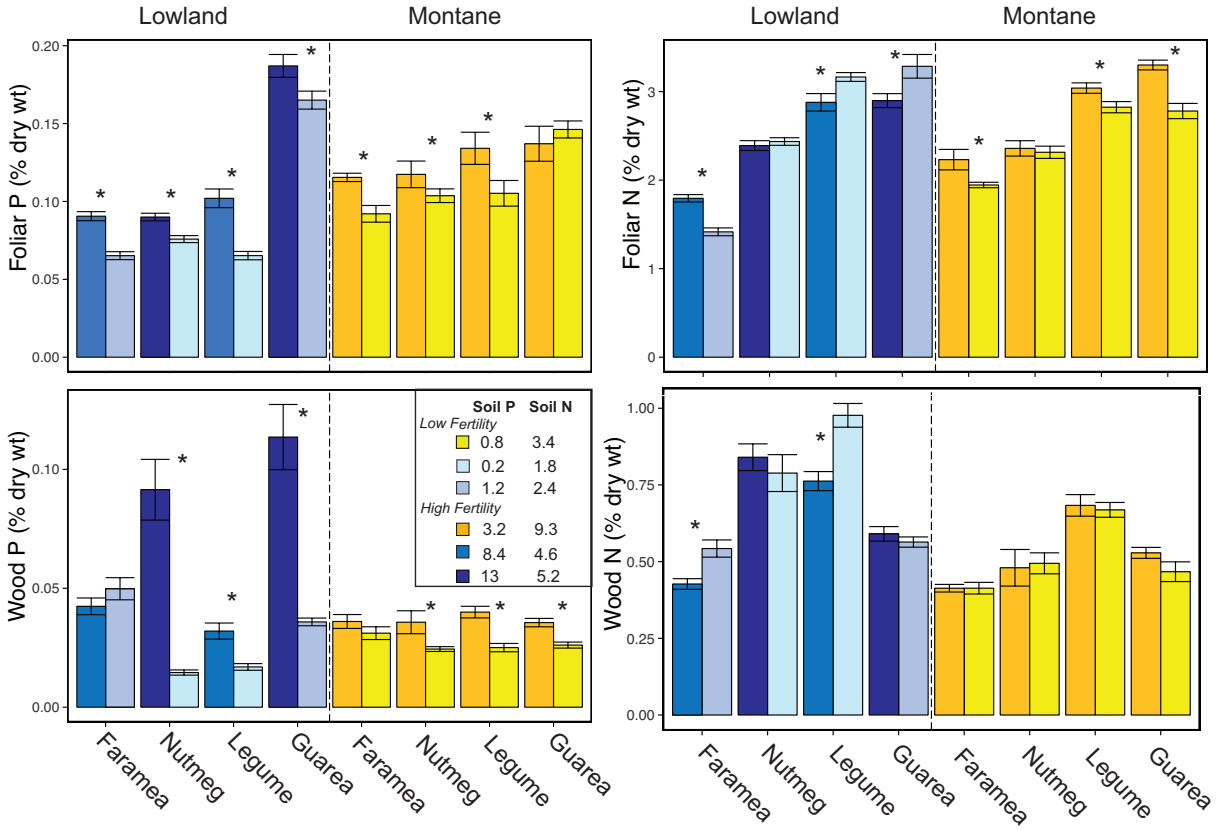


Figure 5.5 Mean remobilization of wood N and P of defoliated saplings from four focal taxa sampled from four habitat types (low and high fertility in lowland and montane forests). Negative values indicate that wood nutrient concentrations significantly declined from time 1 (before defoliation) to time 2 (after leaf reflush response). Y bars represents one standard error.

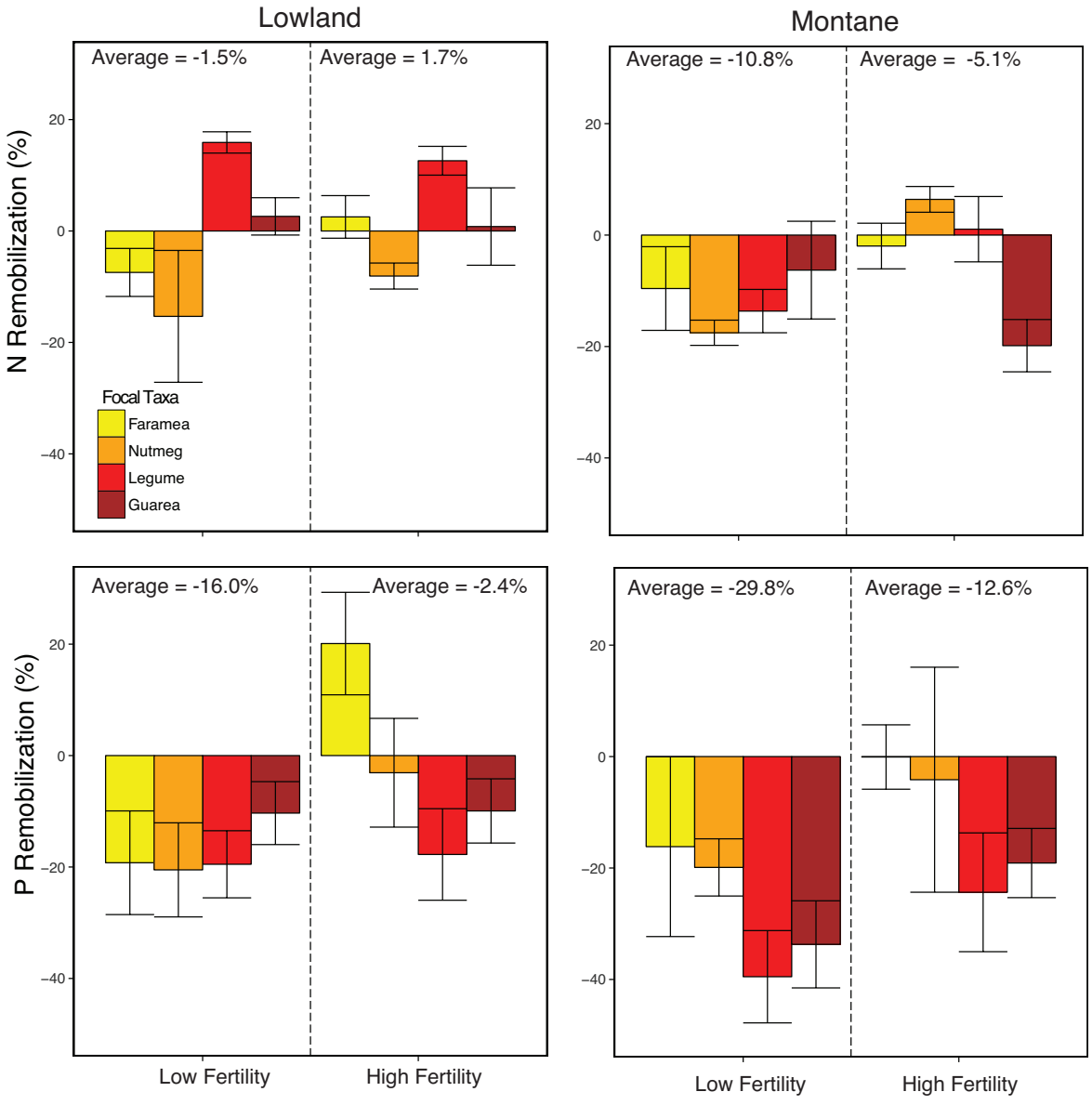
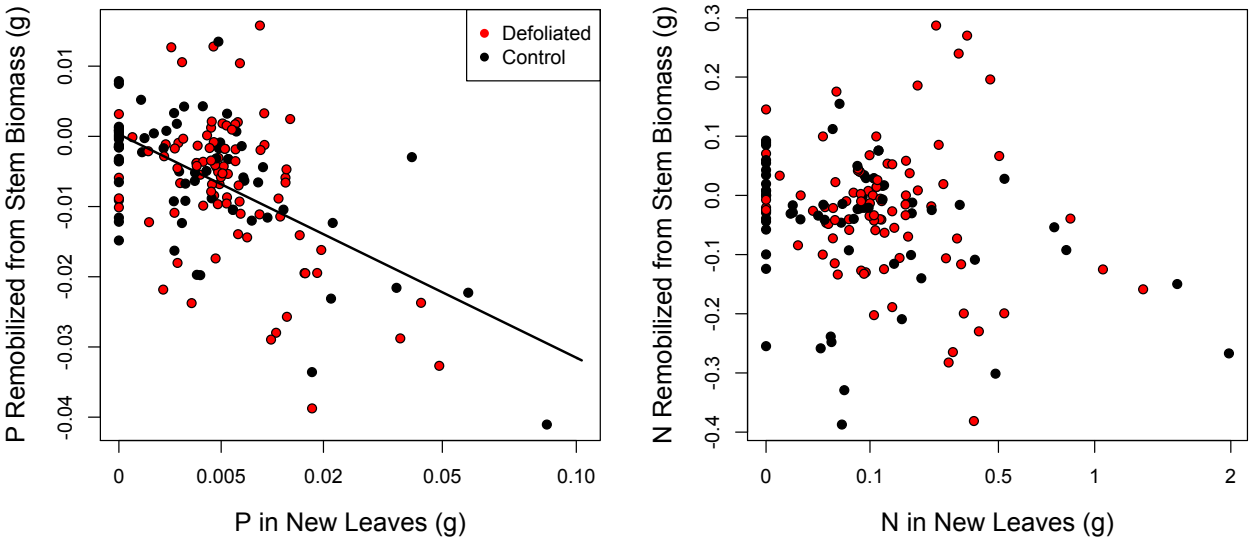


Figure 5.6 Relationship between the quantity of N and P remobilized from stem biomass and the quantity of N and P allocated to leaves reflushed between initial tissue sampling (time 1) and sapling harvest (time 2). Negative remobilization values indicate that N and P concentrations declined from time 1 to time 2. X axes are plotted on square root scales.



CHAPTER 6

CONCLUSION

SUMMARY AND SYNTHESIS

This dissertation research highlights the tremendous variation among tropical tree species in nutrient allocation and emphasizes the importance of wood nutrient storage as a critical aspect of in tropical tree nutrient dynamics. Niche partitioning, enabled by variation among species in resource use, is a primary mechanism by which species coexist in a given environment. However, species may also be physiologically or competitively excluded from environments on the basis of resource, resulting in composition turnover along resource gradients. Therefore, variation in nutrient allocation among tropical species may underlie both the high local diversity in tropical tree communities, and the patterns of soil habitat specialization that are common throughout the tropics (John *et al.*, 2007). Furthermore, because soil nutrient availability is in a continuous feedback loop with the aboveground nutrient allocation (Wardle *et al.,* 2004), understanding how tree community function varies over fertility gradients is critical to estimating rates of ecosystem carbon and nutrient cycling. By discovering the functional importance of wood nutrient storage and remobilization, this work has illuminated previously poorly understood facet of nutrient allocation that has implications for species coexistence dynamics and ecosystem nutrient cycles.

Wood nutrient storage as a functional trait

Wood physical traits, most prominently wood density, have been established as a defining life history of tree species, due to their high interspecific variation and association with

species growth and mortality rates (Chave *et al.*, 2009, Wright *et al.*, 2010). In Chapter 3, I found that tree species also vary enormously among species and sites in wood chemical traits including the concentrations of Ca, Mg, K, N, and P. In particular, species wood P concentrations correlated with several important functional traits, displaying a negative relationship with wood density (Chapter 3) and positive relationship multiple stem frequency (Chapter 4) at Fortuna. The radial decline of P in wood cores in observed in Chapter 3 suggested that P in wood is frequently remobilized. In Chapter 5, we found experimental evidence that plants remobilize a significant fraction wood P reserves to facilitate new tissue creation, especially where soil P is scarce. Wood N content was less variable among species and less sensitive to soil resource availability than wood P, underscoring the qualitative differences in N and P physiology and cycling.

Variation in ecosystem function over edaphic gradients

The incredible variation in plant function observed at the species level strongly influenced ecosystem function over the Panamanian fertility gradients. The analysis of litterfall in Chapter 2 can viewed as a case study in how functional composition of plant communities dominate ecosystem processes, as it demonstrated that two forests growing on similar soil habitats but contrasting in composition can differ by over 60% in canopy productivity. High interspecific variation in wood nutrients observed in Chapter 3 produced four to eight-fold variation among forest sites in community mean wood Ca, K, Mg, N, and P content. Given that wood stores the majority of nutrients in plant biomass, this variation in community mean wood nutrients creates a proportionally generates similarly impressive differences among in total forest nutrient stocks. Finally, the strong correlation between multiple-stem frequency and soil phosphorus availability in Panamanian forest plots indicates that soil nutrients can also influence the gap dynamics and responses to disturbance among tropical forest communities.

SIGNIFICANCE

This work strongly reinforces the notion that functional traits of tree species influence global biogeochemical processes (Cornwell *et al.*, 2008, Cornwell *et al.*, 2009). Specifically, there are a large number of studies seeking to understand if chemistry and decomposition of plant material is conserved across organs (Kerkhoff *et al.*, 2006, Freschet *et al.*, 2013, Pietsch *et al.*, 2014, Zanne *et al.*, 2015). Our finding in Chapter 3 that species mean wood and leaf N, P, K, Mg, and Ca concentrations are correlated among co-occurring tropical tree species, indicates that chemical traits of leave which are available in global plant database such as TRY (Kattge *et al.*, 2011) or potentially estimated from satellite imagery (Kokaly *et al.*, 2009) may be useful predictors of the wood chemical composition and decomposition rates. Furthermore, the publication of comprehensive wood nutrient dataset is an important contribution to the functional traits literature, in which wood trait information is poorly represented.

Furthermore, the insight that tropical trees have dynamic and mobile wood phosphorus reserves has important implications for global carbon models. Feedbacks between carbon and phosphorus cycling in tropical forests are seen as a major source of error in models predicting future global carbon and climate dynamics (Yang *et al.*, 2014). Increasing N-deposition in tropical regions (Hietz *et al.*, 2011) and elevated atmospheric CO_2 concentrations may exacerbate the strength of P-limitation and important of plant-P relations in tropical forests as global change scenarios play out.

FUTURE RESEARCH

I plan to build on my dissertation to develop a more nuanced understanding of tree nutrient storage and its implications for tropical forest biogeochemical cycles. Currently, I am working with Jennifer Jones to characterize within and among differences in bark nutrient

content, which is proving to be an extremely large pool of ecosystem Calcium. I am also analyzing data related to the storage of nutrients in the leaves of *Garcinia* plants in montane forests, which support leaves for up to year after complete coverage by epiphytes. While my next professional project will be focus on the environmental correlates of tree taxa at global scales, I hope to eventually to return to the question of tree phosphorus remobilization in a greenhouse setting where I could test the influence of light, fertilization, and elevated CO_2 on tree resource storage.

LITERATURE CITED

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. **2009**. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**, 351-366.

Cornwell WK, Cornelissen JHC, Allison SD, Bauhus J, Eggleton P, Preston CM, Scarff F, Weedon JT, Wirth C, Zanne AE. **2009**. Plant traits and wood fates across the globe: Rotted, burned, or consumed? *Global Change Biology* **15**, 2431-2449.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**, 1065-1071.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JH. **2013**. Linking litter decomposition of above-and below-ground organs to plant–soil feedbacks worldwide. *Journal of Ecology* **101**, 943-952.

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ. **2011**. Long-term change in the nitrogen cycle of tropical forests. *Science* **334**, 664-666.

John R, Dalling JW, Harms KE, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M et al. 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 864-869.

Kattge J, Diaz S, Lavorel S, Prentice I, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright I. **2011**. Try–a global database of plant traits. *Global Change Biology* **17**, 2905-2935.

Kerkhoff Andrew J, Fagan William F, Elser James J, Enquist Brian J. **2006**. Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *The American Naturalist* **168**, E103-E122.

Kokaly RF, Asner GP, Ollinger SV, Martin ME, Wessman CA. **2009**. Characterizing canopy biochemistry from imaging spectroscopy and its application to ecosystem studies. *Remote Sensing of Environment* **113**, S78-S91.

Pietsch KA, Ogle K, Cornelissen JH, Cornwell WK, Bönisch G, Craine JM, Jackson BG, Kattge J, Peltzer DA, Penuelas J. **2014**. Global relationship of wood and leaf litter decomposability: The role of functional traits within and across plant organs. *Global Ecology and Biogeography* **23**, 1046-1057.

Wright SJ, Kitajima K, Kraft NJB, Reich PB, Wright IJ, Bunker DE, Condit R, Dalling JW, Davies SJ, Díaz S et al. 2010. Functional traits and the growth-mortality trade-off in tropical trees. *Ecology* **91**, 3664-3674.

Yang X, Thornton PE, Ricciuto DM, Post WM. **2014**. The role of phosphorus dynamics in tropical forests–a modeling study using clm-cnp. *Biogeosciences* **11**, 1667-1681.

Zanne AE, Oberle B, Dunham KM, Milo AM, Walton ML, Young DF. **2015**. A deteriorating state of affairs: How endogenous and exogenous factors determine plant decay rates. *Journal of Ecology* **103**, 1421-1431.

APPENDIX

Appendix A Concentration of nutrients in outer 5 cm of sapwood for 106 tree species. Botanical nomenclature based on Correa et al., 2004. Soil, climate, and geographical parameter for each 1-ha forest plot are listed in Table 3.1.

				Conc. outer 5 cm of wood (μg g^{-1})				
Family	Genus	Species	Plot	Ca	K	Mg	N	P
Achariaceae	Lindackeria	laurina	P06	2515	1436	1246	5333	172
Adoxaceae	Viburnum	costaricanum	ChorroA	777	1911	665	1800	84
Anacardiaceae	Mangifera	indica	P13	5866	2029	1318	1550	407
Anacardiaceae	Spondias	radlkoferi	P13	2371	2678	243	2767	324
Anacardiaceae	Tapirira	guianensis	AltoFrio	1786	729	218	NA	101
Anacardiaceae	Tapirira	guianensis	P06	1540	995	307	2133	85
Anacardiaceae	Tapirira	guianesis	P25	2362	1001	408	2200	46
Annonaceae	Annona	pittieri	HondaA	1961	1384	163	3350	124
Annonaceae	Annona	pittieri	Samudio	1496	750	221	3267	82
Annonaceae	Desmopsis	maxonii	Hornito	1986	2323	1157	5800	233
Annonaceae	Guatteria	acrantha	ChorroA	759	1715	811	2733	61
Apocynaceae	Rauvolfia	aphlebia	Hornito	2155	1602	141	2900	69
Apocynaceae	Tabernaemontana	longipes	Samudio	1445	1443	346	3800	122
Araliaceae	Dendropanax	arboreus	AltoFrio	1461	2199	515	1850	123
Araliaceae	Dendropanax	arboreus	HondaA	1010	1696	490	1933	75
Araliaceae	Dendropanax	arboreus	Hornito	1155	2247	518	2467	130
Arecaceae	Colpothrinax	aphanopetala	ChorroA	457	453	266	2333	47
Arecaceae	Euterpe	precatoria	ChorroA	1476	2264	457	1700	67
Arecaceae	Wettinia	quinaria	ChorroA	539	1473	656	2100	31
Arecaceae	Wettinia	quinaria	PaloSeco	1108	779	866	1700	106
Asteraceae	Koanophyllon	hylonomum	Samudio	1210	3044	494	2300	154
Boraginaceae	Cordia	alliodora	P13	4746	749	649	2933	223
Boraginaceae	Cordia	sp1	AltoFrio	2912	3439	564	2300	143
Burseraceae	Bursera	simaruba	P24	2108	1227	1146	3150	668
Burseraceae	Protium	glabrum	P25	1762	727	432	1900	52
Burseraceae	Protium	panamense	P06	1402	1409	415	1833	86
Burseraceae	Protium	tenuifolium	P24	2286	1119	172	1775	99
Calophylaceae	Marila	jefensis	PaloSeco	927	587	195	2300	65
Celastraceae	Zinowiewia	costaricensis	AltoFrio	971	1074	2476	NA	97
Chloranthaceae	Hedyosmum	bonplandianum	ChorroA	1157	2279	730	1767	105
Chloranthaceae	Hedyosmum	bonplandianum	HondaA	815	2899	547	2000	100
Chloranthaceae	Hedyosmum	bonplandianum	Samudio	1593	2120	742	1800	106
Chrysobalanaceae	Hirtella	racemosa	P25	678	526	1334	1933	58

Clusiaceae	Calophyllum	brasiliense	P25	1043	270	240	1300	26
Clusiaceae	Garcinia	madruno	P25	1429	1225	81	2133	62
Clusiaceae	Garcinia	magnifolia	HondaA	1334	668	143	1400	40
Clusiaceae	Symphonia	globulifera	PaloSeco	1207	1559	212	1800	31
Combretaceae	Terminalia	amazonia	P06	2424	529	183	2200	52
Euphorbiaceae	Alchornea	glandulosa	PaloSeco	1484	1405	921	3100	211
Euphorbiaceae	Croton	schiedeanus	HondaA	1317	851	410	2133	69
Euphorbiaceae	Croton	schiedeanus	PaloSeco	2153	1596	610	2933	108
Euphorbiaceae	Croton	schiedeanus	Samudio	1368	1660	660	2433	116
Euphorbiaceae	Euphorbia	elata	PaloSeco	1650	621	975	3550	217
Euphorbiaceae	Hyeronima	oblonga	ChorroA	668	1371	179	1567	51
Euphorbiaceae	Hyeronima	oblonga	HondaA	480	967	236	1200	59
Euphorbiaceae	Pera	arborea	P25	1385	372	219	2333	43
Euphorbiaceae	Tetrorchidium	euryphyllum	PaloSeco	744	1033	159	2750	153
Fabaceae	Inga	alba	Samudio	735	1830	62	NA	22
Fabaceae	Inga	barbourii	PaloSeco	2344	1230	136	3633	185
Fabaceae	Inga	exalata	HondaA	1347	1221	107	3225	39
Fabaceae	Inga	exalata	Samudio	575	637	68	2850	62
Fabaceae	Inga	longispica	AltoFrio	1507	1353	120	2467	53
Fabaceae	Inga	marginata	AltoFrio	1827	1365	91	2600	60
Fabaceae	Pterocarpus	sp1	Samudio	1030	2192	403	3233	116
Fabaceae	Swartzia	simplex	AltoFrio	4014	565	316	3300	52
Fabaceae	Swartzia	simplex	P13	7532	717	243	4767	65
Fabaceae	Swartzia	simplex	P24	5731	2163	209	5200	76
Fabaceae	Tachigali	versicolor	P06	891	816	297	2633	43
Fagaceae	Quercus	insignis	HondaA	1792	558	89	1600	35
Fagaceae	Quercus	sp1	ChorroA	1002	1104	178	1300	38
Icacinaceae	Calatola	costaricensis	HondaA	1338	1370	896	2100	107
Icacinaceae	Calatola	costaricensis	PaloSeco	1073	776	547	2150	107
Icacinaceae	Calatola	costaricensis	Samudio	1249	1544	917	2133	115
Juglandaceae	Oreomunnea	mexicana	AltoFrio	861	1270	467	1633	61
Juglandaceae	Oreomunnea	mexicana	HondaA	1128	855	582	1850	78
Lamiaceae	Aegiphila	panamensis	AltoFrio	1924	2722	367	2600	191
Lauraceae	Lauraceae	sp1	AltoFrio	3833	1115	142	2967	52
Lecythidaceae	Eschweilera	panamensis	Samudio	272	1167	180	2767	73
Lecythidaceae	Gustavia	superba	P13	3367	4825	382	3967	430
Lecythidaceae	Gustavia	superba	P24	3936	1854	636	4650	338
Magnoliaceae	Talauma	sp	HondaA	541	1031	281	2500	58
Malpighiaceae	Bunchosia	macrophylla	PaloSeco	1644	2295	494	3200	122
Malvaceae	Luehea	seemannii	P13	10327	3343	463	2133	235
Malvaceae	Pachira	sessilis	P25	3599	2792	3124	2133	88
Meliaceae	Cedrela	tonduzii	Hornito	2648	1581	231	1750	132
Meliaceae	Guarea	glabra	HondaA	1845	865	201	1900	65
Meliaceae	Guarea	glabra	Samudio	1525	1804	141	2367	89
Meliaceae	Guarea	pterorhachis	Hornito	1278	1379	324	2100	138
Menispermaceae	Hyperbaena	allenii	Hornito	5292	4806	278	5400	154

Metteniusaceae	Metteniusa	tessmanniana	PaloSeco	2936	455	871	1800	78
Monimiaceae	Mollinedia	sp1	HondaA	3025	3275	1031	4150	95
Monimiaceae	Mollinedia	sp1	PaloSeco	1843	2466	535	3800	242
Moraceae	Artocarpus	pusaltilis	P13	1823	2043	645	3150	238
Moraceae	Trophis	racemosa	P13	12613	2053	1162	2900	184
Myristicaceae	Osteophloeum	cf.platyspermum	Samudio	932	900	282	2100	107
Myristicaceae	Virola	sebifera	P06	2688	2860	384	2950	72
Myristicaceae	Virola	sebifera	P13	922	776	260	3900	170
Myristicaceae	Virola	sebifera	P25	705	697	242	4167	54
Myrtaceae	Eugenia	sp3	Hornito	5555	1613	372	2450	111
Myrtaceae	Plinia	sp1	Samudio	878	1223	484	1700	71
Podocarpaceae	Podocarpus	oleifolius	ChorroA	847	752	128	1667	51
Polygonaceae	Coccoloba	obovata	AltoFrio	10861	4531	922	2500	130
Polygonaceae	Triplaris	cumingiana	P24	7444	1632	124	3833	97
Primulaceae	Cybianthus	montanus	ChorroA	417	1779	146	1800	54
Proteaceae	Roupala	montana	Hornito	999	1151	297	1333	43
Putranjivaceae	Drypetes	brownii	PaloSeco	2262	2391	1303	3567	224
Rhizophoraceae	Cassipourea	elliptica	HondaA	4108	1351	139	2767	73
Rhizophoraceae	Cassipourea	elliptica	P25	2657	935	448	2667	59
Rhizophoraceae	Cassipourea	elliptica	Samudio	1619	1053	239	2400	82
Rosaceae	Prunus	brachybotrya	AltoFrio	1206	1795	212	1400	114
Rosaceae	Prunus	cf fortunensis	AltoFrio	1667	1025	459	1900	122
Rubiaceae	Alibertia	garapatica	Samudio	1111	1437	362	2367	70
Rubiaceae	Alseis	blackiana	P06	2074	1093	389	3567	140
Rubiaceae	Amaioua	pedicellata	HondaA	926	1717	155	2767	56
Rubiaceae	Amaioua	pedicellata	PaloSeco	605	886	149	2850	54
Rubiaceae	Chomelia	sp1	Hornito	725	2424	222	4200	279
Rubiaceae	Elaeagia	auriculata	HondaA	719	2246	248	2267	68
Rubiaceae	Faramea	multiflora	Samudio	2101	2824	786	3250	101
Rubiaceae	Faramea	occidentalis	P24	3358	2247	409	2650	131
Rubiaceae	Joosia	umbellifera	PaloSeco	663	1897	536	2967	116
Rubiaceae	Palicourea	roseofaucis	HondaA	644	2085	306	1850	34
Rubiaceae	Palicourea	roseofaucis	Samudio	589	2871	364	1933	58
Rubiaceae	Pentagonia	nuciformis	PaloSeco	2745	1592	869	2100	119
Rubiaceae	Pentagonia	nuciformis	Samudio	2273	2599	978	2950	119
Rubiaceae	Pittoniotis	trichantha	P24	1791	3169	919	2700	194
Rubiaceae	Posoqueria	latifolia	ChorroA	1482	685	268	1967	33
Rubiaceae	Posoqueria	latifolia	HondaA	1190	936	447	2000	56
Rubiaceae	Posoqueria	sp1	PaloSeco	985	578	331	2933	101
Rubiaceae	Psychotria	luxurians	ChorroA	1297	3039	126	1900	48
Rubiaceae	Psychotria	panamensis	Hornito	1337	5474	150	2433	129
Rutaceae	Peltostigma	guatemalense	Hornito	3983	2538	731	4075	100
Sabiaceae	Meliosma	allenii	HondaA	1123	1486	450	1633	65
Salicaceae	Hasseltia	floribunda	Hornito	1617	5772	959	4000	226
Sapindaceae	Billia	rosea	Hornito	908	679	121	1400	64
Sapindaceae	Cupania	scobiculata	P06	5918	987	1948	2000	100

Sapindaceae	Cupania	scrobiculata	P25	1654	1206	1035	2050	55
Sapindaceae	Matayba	apetala	P25	922	399	276	1900	38
Sapotaceae	Chrysophyllum	argenteum	AltoFrio	1560	1969	426	2733	109
Sapotaceae	Manilkara	chicle	AltoFrio	2715	645	188	1850	33
Sapotaceae	Micropholis	melinoniana	ChorroA	1909	2294	478	2250	75
Sapotaceae	Micropholis	melinoniana	HondaA	1061	1184	268	1667	72
Sapotaceae	Micropholis	melinoniana	Samudio	778	1404	482	2300	116
Sapotaceae	Pouteria	cuspidata	HondaA	400	901	126	1467	28
Sapotaceae	Pouteria	juruana	AltoFrio	1214	1424	904	3167	104
Sapotaceae	Pouteria	juruana	Hornito	1191	2268	1213	3000	146
Sapotaceae	Pouteria	reticulata	PaloSeco	881	2377	206	2750	164
Symplocaceae	Symplocos	limoncillo	AltoFrio	855	993	318	1500	40
Tapisciaceae	Turpinia	occidentalis	AltoFrio	1150	2696	1812	2167	NA
Vochysiaceae	Vochysia	ferruginea	P06	869	1251	551	2100	51
Vochysiaceae	Vochysia	guatemalensis	HondaA	308	171	170	1533	21
Vochysiaceae	Vochysia	guatemalensis	Samudio	383	143	186	1567	18